EFFECTS OF ULTRA-HIGH TEMPERATURES ON BACILLUS SUBTILIS VAR. NIGER SPORE AEROSOLS AS A FUNCTION OF RELATIVE HUMIDITY

by

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TABLE OF CONTENTS

		page
I.	INTRODUCTION	1
II.	REVIEW OF LITERATURE	4
	A. Early Investigations in Thermal Sterilization	4
	B. The Nature of Thermal Sterilization	5
	C. Effects of High Temperatures on Bacterial Spores	8
	D. Effects of Moisture Level on Dry Heat Resistance of Bacterial Spores	15
	E. Sublethal Heat Injury of Bacterial Spores	18
III.	MATERIALS AND METHODS	22
	A. General Procedures for Obtaining Time/Temperature Relationships	22
	B. Preparation of Spore Crops	23
	C. Production of Calibrated Spore Aerosols	25
	D. The Incineration Test System	30
	1. Humidity Control	30
	2. Environmental Monitoring	33
	3. Syringe Injection Port	34
	4. The Incinerator	34
	5. Gas Cooler	37
	6. Air Sampler	37
	7. Vacuum Pump	38
	E. Procedures Used for Experiments in Time/Temperature Relationships	38
	F. Procedures for Sublethal Heat Injury Studies	42
	G. Data Reduction and Analysis Procedures	43
	1. Preliminary Data Reduction	43
	2. Final Data Reduction	44
	3. Heat-Injury Calculations	50

IV. RESULTS 56

A. Results of Preliminary Calculations 56

B. Results of Final Data Reduction 60

C. Heat Injury Experiments 65

V. DISCUSSION 70

A. D Values 70

B. Z Values 74

C. Transition Points 75

D. Effects of Relative Humidity 78

E. Optimizing the Biological Efficiency of Incinerators 82

F. Corrected D and Z Values 82

G. Sub-lethal Heat Injury Effects 84

VI. SUMMARY AND CONCLUSIONS 87

LITERATURE CITED 90

APPENDIX 95

VITA 100

INTRODUCTION

Traditionally the three primary methods utilized to sterilize or decontaminate gases have been filtration, ultra-violet irradiation, and thermal irradiation. All of these methods, compared in detail by Cherry, et al. (1969), have one or more economic, practical, or biological disadvantages. In short, thermal sterilization of flowing gases is usually considered the least practical of the three choices from the equipment reliability and economy viewpoints, but is often judged the best from the viewpoint of biological effectiveness. It seems, therefore, that thermal sterilization of gases, often called incineration, would be used more often if a more durable and economic approach were developed. One promising method for overcoming these disadvantages is the use of compressors to generate the required heat. However, there is some doubt that compressors can be designed to generate the high "instantaneous" temperatures that are required to obtain sterility in many situations (Cherry, et al. (1969).

An efficient, reliable, and economic incinerator system would have a number of potential applications, both in the food industry and in fields such as public health and pharmaceutical manufacture. These applications can be grouped according to whether supply or exhaust gas systems are involved. Some examples of the latter might include poultry processing plants where both high bacterial counts and high levels of suspended particulate matter (which tends to plug filters) are common, and public health research facilities where high numbers of pathogenic organisms must be removed from gases before

they can be exhausted from the laboratories. Dairy plants, which are often the first to utilize advances in microbial contamination control, would have applications in the area of general environmental control, and in the area of powdered milk production.

One area in the food industry which has already indicated interest in an improved incinerator system is aseptic canning. At present, super-heated steam is being utilized in aseptic cannering equipment to sterilize cans and lids, and to provide a sterile environment in which the can filling can be carried out. Steam, however, has some definite limitations: (1) pressure is required to maintain sterilization temperatures; (2) trapped air and dissolved oxygen in the steam provide sources of oxygen which can lead to oxidation reactions during subsequent storage of the food product and result in off flavors and odors; and (3) the moisture in steam restricts the use of aseptic canning largely to moist or wet products. However, hot, dry nitrogen gas, sterilized by passing it through an incinerator system, could be used instead of steam, allowing an unpressurized system which would contain no oxygen or moisture. Based on the results of this investigation an incinerator outlet temperature of 450°F(232°C) should be sufficient for this application.

The primary stimulus to this investigation was the development by the author, in conjunction with Mr. Richard M. Hueschen of the Langley Research Center, NASA, of a highly reliable electric incinerator which is capable of temperatures up to 1100°F(593°C). Further incentive was provided by test data which indicated that the higher

the temperature of the incinerator, the more economically it can be operated. Thus a potential combination of factors had become available which, based on reliable data, could lead to increased use of incineration for the sterilization of gases. However, a review of the pertinent literature showed that while research on the effects of high temperatures on airborne microorganisms had been reported, the results were generally in disagreement and that temperatures greater than 675°F(357°C) had not been investigated. It was further discovered that the effects of the moisture content of the carrier gas had not been investigated at these temperatures. There was obviously a need for further microbiological research in this area.

The objective of this investigation were to determine: (1) the mathematical relationship between the exposure time required to give a 90 percent reduction in a known gas-borne microbial population (D value) and the temperature over a range of 300-1100°F(149-593°C); (2) the effect of variations in the moisture content of the carrier gas on this time/temperature relationship; and (3) the role, if any, that sub-lethal heat injury plays in the apparent population reductions observed at various temperatures. These parameters were selected for study because of their important roles in determining the effectiveness of any thermal sterilization technique. It is hoped that the availability of reliable data on these parameters will lead to future studies of other related parameters.

REVIEW OF LITERATURE

A. Early Investigations in Thermal Sterilization

Studies on the effects of heat as a preserving or sterilizing agent began late in the eighteenth century with the efforts of Spallanzani, who was not only able to show that heating of infusions could free them from growing microbes, but also to differentiate between large microbes, which could be destroyed by boiling for one-half minute, and "microbes of the lowest order" which often survived boiling and which began to grow in his sealed containers within a period of a few days. The latter description may well have been the first for bacterial spores. Spallanzani's results were repeated in a very practical sense by Nicholas Appert in the very early nineteenth century when he showed that boiling of fruits and vegetables in sealed containers preserved them almost indefinitely (Thimann, 1963).

It is interesting to note that many of the early experiments in bacteriology were performed with airborne organisms. This was particularly true of those of Schultze in 1836, Schwann in 1837, and Schroder and Dusch in 1853. However, the most dramatic of these experiments, and the one which is most closely related to the subject of this dissertation, was that of Louis Pasteur in 1862. Pasteur boiled infusions in a long necked vessel which was connected by a short tube wrapped with a wet cloth to an apparatus which was essentially a gas "incinerator". The boiled infusions were allowed to cool, while the entering air was heated then cooled before it reached

the flask. Under these conditions the infusion remained clear, with no growth of microorganisms. By this experiment Pasteur confirmed the conclusions of Schwann and others that the recontamination of the boiled infusions in earlier experiments had not been due to spontaneous generation, but rather to "germs...which...are present in the air, (and) are destroyed by burning out the air." This was probably the first demonstration of the sterilizing capability of the incinerator as we know it today. An interesting resume of the work of the early investigators can be found in the book by Thimann (1963).

B. The Nature of Thermal Sterilization

Thermal sterilization processes have by convention been divided into two types, wet (when organisms are suspended in water solutions or when saturated steam is used as a heat source) and dry (when any other type of convective or conductive heating is used). Wet heat causes the irreversible denaturation of enzymes and nucleic acids critical to the survival of the organisms (Rahn, 1945). This theory has been extensively supported by thermodynamic studies (Cox and Peacocke, 1957; and Cavalieri and Rosenberg, 1957). Perhaps the most revealing data on this subject is that the Q_{10} (the ratio of the rate constants of a reaction at two temperatures differing by ten degrees Centigrade) for the wet heat inactivation of bacterial spores, which is exceptionally high and lies between 10 and 20, is about the same as the Q_{10} for protein denaturation by wet heat (Thimann, 1963).

The mechanism of "dry" heat inactivation is not so clearly

defined or generally accepted as that for wet heat, perhaps because "dry" heat is itself not clearly defined. The contention that macromolecule denaturation is not the mechanism of "dry" heat inactivation is supported by the results of Rowe and Silverman (1970), who found no differences between the water absorption/desorption isotherms of dry heat inactivated and viable spores of Bacillus subtilis. This indicates that dry heat inactivation does not result in drastic structural alteration such as found in wet heat inactivated spores. However, since the Q_{10} for "dry" heat inactivation of bacterial spores is usually in the range of 2-4, the mechanism is obviously not the same as for wet heat (Rahn, 1945). These much lower values are commonly associated with "simple" chemical reactions, which tends to support the general theory that "dry" heat inactivation is due to progressive desiccation and oxidation of critical cell components.

It appears that under conditions of low relative humidity, the "dry" heat mechanism predominates, while at high humidities the inactivation follows the pattern of wet heat sterilization. Pflug and Schmidt (1968) said "The boundary between wet heat death of microorganisms and dry heat destruction may be at a water activity of about 0.9". In air this value is equivalent to a relative humidity of 90%. The effect of relative humidity on "dry" heat inactivation will be discussed in detail in another section of this review.

The thermal death of a genetically, chemically, and physically uniform population of bacteria occurs according to a logarithmic order. This was observed early in this century by Chick (1908), who

also reported that this phenomenon was due to a monomolecular (first-order) reaction within the cells. This observation has since been made by countless researchers over a greatly varying range of organisms, temperatures, and environmental conditions (Rahn, 1945; Pflug and Schmidt, 1968).. This relationship can be observed by plotting the log of surviving population against time, producing a plot commonly known as a survivor curve. The slope of the survivor curve is a function of the innate heat resistance and the physiological state of the organism, the temperature, the humidity of the environment, the recovery medium, and the incubation temperature, as well as a number of factors of lesser impact. The slope of the survivor curve, i.e., the death rate of the population, is usually represented by a death rate constant called the "D-value". The D-value is simply the time required to reduce the number of survivors by 90% or the log of the population by one. When a D-value for a particular organism is presented in the literature, it must be accompanied by the temperature and as much other data about the environmental conditions as possible.

The D-value for an organism under wet heat conditions differs very significantly from the D-value obtained under "dry" heat conditions at the same temperature. For example, the D_{266^oF} for Bacillus stearothermophilus spores deposited on paper in a dry heat oven is approximately 720 times as great as when they are suspended in phosphate buffer (Bruch, 1966; Wang et al., 1964). There is also a very significant difference between the heat resistance of the spore and

vegetative cell forms of the same bacterium. Further discussion will be limited to "dry" heat conditions and bacterial spores. A good review of the factors effecting thermal death of bacteria under wet heat conditions is provided by Pflug and Schmidt (1968).

C. Effects of High Temperatures on Bacterial Spores

Data on the effects of "dry" heat on bacterial spores was relatively scarce in the first half of this century, particularly when compared to that available on the effects of wet heat. In addition, Rahn pointed out that in 1945 "a great deal of confusion exists with regard to the proper time-temperature relationships for sterilization by dry heat". This confusion was due to the unrecognized influence of relative humidity and other environmental factors on the death rate of spores, and the lack of data was primarily due to the limited uses to which "dry" heat sterilization had been applied. In the 1950's and 1960's a better understanding of the effects of environmental factors stimulated research and led to better, more compatible data. A stimulus to research in this area was applied by the decision of NASA in the early 1960's to utilize "dry" heat to sterilize interplanetary spacecraft operating under planetary quarantine constraints (Bruch, 1968). The resulting research has provided an adequate body of data which covers the high-temperature range, from 221°F(105°C) to 275°F (135°C), with some data extending to 320°F(160°C). The most complete data are available for 248°F(120°C) and 257°F(125°C). Excellent reviews of these data are available (Bruch, 1968; Pflug and Schmidt,

1968; and Russell, 1971). However, in the ultra-high temperature range above 320°F(160°C), the available data become more scarce and conflicting in some cases, particularly when extrapolations are necessary to compare data. At the present time it appears that ultra-high temperature "dry" heat research is at about the same stage as was high temperature research in the early 1950's.

Before discussing this research in detail, it is first necessary to review the contribution of two important "environmental" factors which complicate the comparison of extrapolations of high temperature data with ultra-high temperature data, that is, the effect of carriers or substrates and the mutually protective effects of large populations of bacterial spores. The effect of substrates can usually be related to the heat lag associated with heating the surface, if that is the source of the heat, or the heat conductivity of the surface if the surrounding gas is the source of heat. Bruch, et al. (1963) found that spores of B. subtilis var. niger deposited on paper strips gave consistently lower D-values than the same organisms deposited on glass, while Paik, et al. (1969) reported a significant difference between D-values obtained when stainless steel was used as a substrate, and when conversion-coated magnesium was used. Obviously the comparison of data from experiments using different substrates is questionable. This source of error is further compounded when data compiled using any substrate is extrapolated and compared with data obtained when no substrate is used, as is the case with ultra-high temperature investigations utilizing incinerators.

A further complicating factor is that a layering phenomenon is obtained when spores from a suspension are deposited on a surface. This layering provides mutual protection against desiccation and heating within the spore population. This phenomenon does not occur to any significant extent when aerosols of spores are used as the test population, as is the case with the high temperature studies. Thus, it appears that in only very isolated situations can existing data from the high temperature range be compared with ultra-high temperature data, and to extrapolate such data to ultra-high temperatures would introduce considerable error. Therefore, reference data from ultra-high temperatures should be used whenever possible.

The first known report of quantitative data relating to the time required to sterilize bacterial spore aerosols at high temperatures came from Bourdillion et al. (1948). These investigators tested two types of electrically heated furnaces, the "externally heated" and the "internally heated", and used both wet and "dry" spores of Bacillus subtilis as test organisms. Some D-values calculated from their data were: At 302°F(150°C) wet spores in the "internally heated" furnace, wet spores in the "externally heated" furnace, and dry spores in the "externally heated" furnace, gave D-values of 8.3, 1.0, and 0.6 sec. respectively, while at 392°F(200°C) the same conditions gave D-values of 4.1, 0.26, and 0.32 sec. respectively. Bourdillion, et al. concluded that the difference noted between furnace designs were at least in part due to a difference in the intensity of thermal radiation produced in the furnaces, but did not comment on the differences noted

between wet and dry spores. Further analyses of their data can be found in the DISCUSSION Section. These data were being used as criteria for the design of industrial and special purpose incinerators as recently as 1970.

Decker et al. (1954) found that using an electric grid air sterilizer they could obtain 99.9999% reduction of an aerosolized population of Bacillus globigii (now B. subtilis var. niger) spores with the following temperature/time relationships: 425°F(218°C)/24 seconds; 475°F(246°C)/10 seconds; 525°F(274°C)/5 seconds; and 575°F(302°C)/3 seconds. These data can be used to calculate D-values of 3.0; 1.7; 0.8; and 0.5 seconds respectively. These researchers concluded that over the range of temperatures they investigated that a 50 degree F increase in temperature is approximately as effective as tripling the exposure time. It should be noted that neither Bourdillion et al. or Decker et al. reported the relative humidities of their systems, although Decker et al. used a wet nebulizer to produce their aerosols and they did mention that relative humidity data were obtained.

Gremillion et al. (1958) reported that temperatures of 400°F (204°C) and above would sterilize a population of 1×10^3 spores of Bacillus subtilis var. niger per cubic foot of air. They also used a wet nebulizer to obtain an aerosol and their incinerator was also of the internally heated electric type, although of a design different from that of Decker et al. It is possible to calculate only one D-value from their results, that being at 350°F(177°C) where D equalled

0.038 seconds. This value is approximately 1/100 of the D values reported for this temperature by Bourdillion et al. (1948), and Decker et al. (1954) using internally heated systems. This investigation was followed by Harris et al. (1964) who essentially repeated much of the work of Gremillion et al., but used different flow rates, and a slightly different incinerator design. Their data cannot be used to calculate D-values, but they did obtain complete sterilization of 4.8×10^{10} and 3.0×10^{11} spores (wet) of B. subtilis var. niger 360°F(182°C) and 402°F(205°C) respectively. Exposure times were not given.

More extensive data were provided by Elsworth et al. (1961) who reported experimentally determined time/temperature data in the range of 212°F to 527°F(275°C). Some D-values calculated from their data are: 1.13 seconds at 392°F(200°C), and 0.36 seconds at 482°F(250°C). Extrapolation of their data to 572°F(300°C) gives a "D-value" of 0.16 seconds. Elsworth et al. used Bacillus globigii spores as test organisms and an internally heated, electrical incinerator with a single, centrally located thermocouple. Neither the water content of the organisms nor of the air carrier were given.

In 1968, Barbeito et al. reported on the most complete investigation of high temperature effects that had yet been undertaken. These investigators studied both wet and dry spores of B. subtilis var. niger as well as dry vegetative cells of Serratia marcescens. Their incinerators were high flow (1000-2200CFM) and used fuel oil burners as heat sources. Temperatures were monitored at several locations

including heat-up and cool-down. Some D-values calculated from their data are: 0.178 seconds at 500°F(260°C), and 0.137 seconds at 600°F (316°C) for wet spores of B. subtilis and 0.113 seconds at 675°F (357°C) for dry spores. The two most recent investigations in this area were those of Chatigny et al. (1970) and Mullican et al. (1971). The former workers utilized a high flow, gas heated incinerator, similar to those used by Barbeito et al., which was capable of flow rates up to 550CFM. Using wet aerosols of B. subtilis var. niger, they obtained the following temperature/D-value relationships: 350°F (177°C)/2.32-4.9 sec., 400°F(204°C)/1.61-2.92 sec., 450°F(232°C)/0.72-1.48 sec., 500°F(260°C)/0.55-1.17 sec., and 550°F(288°C)/0.263 seconds. The heat-up and cool-down of the heated air was well monitored in these experiments. Mullican et al. (1971) reported tests of a laboratory model, electrically heated incinerator. This model was externally heated and the spores did not come in contact with the heating elements. Using wet (23% R.H.) and dry (10% R.H.) aerosols of B. subtilis var. niger they were able to obtain complete sterilization of 1×10^{10} organisms in 0.02 sec. at temperatures of 446°F(230°C) and 500°F(260°C) respectively. These authors were the first and only workers to report carrier gas relative humidities.

Due to the great variations in incinerator design, temperature profiles, carrier gas humidities, recovery and growth techniques, and data analysis procedures, it is almost impossible to make any comparisons among the data presented by the above researchers. An examination of Table 1 will give some indications of the problems

TABLE 1

Variation of Parameters Among Researchers

REFERENCES	Incinerator Design Factors: HEAT SOURCE (Electric, gas, or oil)	Incinerator Design Factors: METHOD OF HEAT TRANSFER TO SPORES	Incinerator Design Factors: DOES SPORE CHAMBER CONTACT WITH HEAT SOURCE?	Incinerator Design Factors: NUMBER OF THERMOCOUPLES USED	Incinerator Design Factors: ARE THERMOCOUPLES EFFECTED BY DIRECT RADIATION?	Incinerator Design Factors: MEAN FLOW RATE (in CFM)	Nature of Challenge Organisms: NAME OF TEST ORGANISM	Nature of Challenge Organisms: GROWTH CONDITIONS, MEDIUM (TEMP.)	Nature of Challenge Organisms: WET OR DRY SPORES	Nature of Challenge Organisms: WERE SPORES HEAT-SHOCKED BEFORE USE?	Nature of Challenge Organisms: AGE OF SPORES (in months)/ DURATION	Nature of Challenge Organisms: METHOD USED TO PRODUCE SPORE AEROSOL	Recovery and Growth Factors: RECOVERY TECHNIQUE FOR SPATTERS	Recovery and Growth Factors: RECOVERY/GROWTH MEDIA	Recovery and Growth Factors: INCUBATION TEMPERATURE IN (°C)	Recovery and Growth Factors: WAS HEAT INJURY INVESTIGATED?
Bourdillon et al., 1948	Elec.	RAD. & CON.	Yes/ No [3]	1	No	20-60	B.s.	Unk.	Both	Yes	Unk.[5]	Neb.	S.S.	HBSA	37	No[4]
Decker et al., 1953	Elec.	RAD. & CON.	Yes	6	No	10-75	B.g.	Unk.	Wet	Yes	Unk.	Neb.	Imp.[1]	NuA	Unk.	No
Gremillion et al., 1958	Elec.	RAD. & CON.	Yes	1	No	1	B.n.	CDM (Unk.)	Wet	Yes	Unk.	Neb.	Fil.	CSA	37	No
Ellsworth et al., 1961	Elec.	RAD. & CON.	Yes	1	No	3.6	B.g.	Unk.	Dry	Unk.	Unk.	Neb.	Fil.& S.S.	Unk.	Unk.	No
Harris et al., 1964	Elec.	RAD. & CON.	Yes	1	Yes	16 & 100	B.n.	Unk.	Wet	Yes	Unk.	Neb.	Imp.[1]	CSA	37	No
Barbeito et al., 1968	Oil	RAD. & CON	Yes	7	1-Yes 6-No	1000& 2200	B.n.	Unk.	Wet& Dry	No	Yes	Neb./ Dis.[2]	Fil.	CSA	37	No
Chatigny et al., 1970	Gas	RAD.& CON.	Yes	7	2-Yes 5-No	550	B.n.	Unk.	Wet	Yes	Unk.	Neb.	Fil.	TSA	35	No
Mullican et al., 1971	Elec.	CON.	No	3	No	0.5	B.n.	Unk.	Wet& Dry	No	Yes	Neb./ Dis.[2]	Imp.	H_2O/ PCA	35	No

KEY TO ABBREVIATIONS

B.S. - Bacillus subtilis
S.S. - Slit sampler
RAD. - Radiation
CON. - Convection/Conduction
B.n. - Bacillus subtilis var. niger
B.g. - Bacillus globigii (old name for B.s.)
TSA - Trypticase soy agar
CSA - Corn steep agar
PCA - Plate count agar
Fil. - Filter technique
D.P. - Direct plating
Imp. - Impinger (AGI)
Neb. - Nebulizer
Oth. - Other
Unk. - Unknown
NuA - Nutrient Agar
HBSA - Hartley broth serum agar
CDM - Casein Hydrolysate Med.

NOTES: [1]Solid medium impinger/sieve sampler. [2]Dried spores were disseminated by agitating a spore powder with a high speed air stream. [3] Difference in radiation level. [4]owever, heat injury was indicated by data, but authors did not recognize it. [5]Dry spores were stored for 11-12 days before use.

inherent in any such comparisons. An example of the variation of data presented can be obtained by an observation of the D-values presented for 350°F(177°C) which range from 0.038 seconds (Gremillion et al., 1958) to approximately 15 seconds (Decker et al., 1954) with many others in between. However, some limited comparisons of these data will be attempted in the DISCUSSION Section.

D. Effects of Moisture Level on Dry Heat Resistance of Bacterial Spores

During the last 20 years it has become firmly established that the moisture content of spores and the humidity of their gaseous environment directly affect their heat resistance (Marshall et al., 1963; Murrell and Scott, 1966; and Angelotti et al., 1968). As mentioned earlier in this review, the heat resistance of spores at 100% relative humidity (R.H.) can be only 1/700th of spores equilibrated at much lower R.H.'s. It has been reported by Anderson (1959) that under conditions of saturated moisture content the transfer of moisture from the atmosphere to the spores is so rapid that even dry spores act as if suspended in water. This effect also evidently occurs at lower relative humidities, as Rowe and Silverman (1970) indicated. They found that spores of B. subtilis which were initially at a water activity less than that of their environment absorbed water rapidly at 242°F(116°C) and equilibrated at the water content of their environment. Thus, the moisture content of the heating environment is probably of greater importance than the initial moisture content of

the spores.

Recent research in this area has emphasized both the quantitation of humidity effects at less than saturation levels, and the basic mechanism of the effect. Present concepts in the latter area are based on the hypothesis of Black and Gerhardt (1962) that the "core of the dormant spore exists as an insoluble and heat-stable gel, in which cross-linking between macro-molecules occurs through stable but reversible bonds so as to form a high polymer matrix with entrapped free water". The proven critical role of moisture in the heat resistance of "dry" spores suggests that at least some of the cross-linking is due to water molecules (Pflug and Schmidt, 1968). Gilbert, et al. (1964) carried this theory one step further by speculating that 'When all or almost all of the (bridging) water molecules are removed, direct cross-linkage (between or within macro-molecules) could be established in their place, blocking what might otherwise by alkylation sites". Although this theory explains the increase in heat resistance noted as the moisture content is decreased from saturation to a water activity (a_W) of around 0.5, it does not relate to the subsequent decrease in heat resistance noted as the moisture content decreases below that point (Murrell and Scott, 1966; Angelotti, et al., 1968; and Brannen and Garst, 1972). Brannen (1971), however, suggested that beyond some point (probably between a_W of 0.3 and 0.5) the additional loss of water molecules from the spore exerts an unstabilizing influence on the macro-molecules themselves which leads to their increased heat sensitivity at low a_W's. He supports this contention

by a kinetic analysis of the data of Angelotti et al. (1968). It appears, therefore, that two mechanisms may be involved in the observed effects of spore moisture content on heat resistance; one which causes increased heat resistance with decreased moisture content, and another which has the reverse effect. This relationship would result in the biphasic curve noted by so many researchers.

As previously pointed out, dry heat research has been largely limited to temperatures of 320°F(160°C) and below. This limitation is reflected in the efforts made to obtain quantitative data on the effects of spore moisture content. Marshall, et al. (1963) used 185°F (85°C), while Fox and Eder (1969) used 203°F(95°C), 235°F(113°C), 264°F(129°C), and 305°F(152°C). Brannen and Garst (1972) used 221°F (105°C), 239°F(115°C), and 257°F(125°C), while Angellotti et al. (1968) investigated temperatures between 221°F(105°C) and 320°F (160°C). In spite of the variation in temperatures used these investigators reported very similar results, with only some displacement of the point of maximum heat resistance being noted. Only two reports of work at ultra-high temperatures, above 320°F(160°C), have been noted. Hoffman et al. (1968) reported data at temperatures of 327°F (164°C) and 378°F(192°C), and relative humidities of 11, 33, and 85%. Although they obtained conflicting results with two strains of B. subtilis var. niger, that is, the maximum heat resistance with strain A was at an R.H. of 85%, while that for strain B was at 11%, they did observe that these effects became less pronounced as the temperature was increased. At a temperature of 378°F(192°C) neither strain

exhibited any significant relationship between heat resistance and R.H. levels. Mullican et al. (1971), however, noted a distinct difference between the temperature required to sterilize a population of wet (23% R.H.) B. subtilis var. niger (446°F/230°C) and that required for a population of dry (11% R.H.) spores of the same organism (500°F/260°C).

An additional relationship between heat resistance at various a_W's and temperature is revealed if the data of Murrell and Scott (1966), and Angelotti et al. (1968) are plotted together. Over a range between 212° and 275°F(135°C), a definite trend was observed where an increase in temperature was accompanied by a decrease in the a_W of maximum heat resistance. Unfortunately, data at higher temperatures were not sufficient to determine if this effect is continuous.

E. Sublethal Heat Injury of Bacterial Spores

Although there was some early indirect evidence that sublethal wet heat injury occurred in bacterial spores as well as vegetative cells (Curran and Evans, 1937), it was not until the 1950's that these effects were actively investigated. Amaha and Sakaguchi (1957) reported "that the death of bacterial spores by heat is a gradual chemical process which proceeds step by step with the time of heating and (which) can be reversed, at least in the first stage, under the proper conditions of subculture". More recent investigations have indicated that this injury can be manifested in a number of ways. Edwards et al. (1956b), using a B. subtilis spore population which

had been heated for 2.7 sec. at temperatures between 245°F9118°C) and 265°F(129°C), noted higher counts of survivors on a fortified nutrient agar incubated at 32°C than at 45°C. The opposite effect was noted with unheated controls and survivors of heating at temperatures below 245°F. They also reported that significant reductions in survivors were observed on a fortified nutrient agar in relation to those noted on the same medium plus $CaCl_2$ and sodium dipicolinate. Maximum heat injury was noted at a temperature of 250°F(121°C), with a disappearance of the effect noted at 275°F(135°C). Edwards et al. did not, however, note any increases in survivor counts when incubation times exceeded 18 (@45°C) or 70 (@32°C) hours. In the same year, Campbell et al. reported that heat (250°F/121°C) injured spores of B. stearothermophilus were unable to grow on a minimal medium unless certain amino acids were added.

More recently the above results have been supported and expanded by Adams and Busta (1972), who identified protein denaturation as the mechanism of wet heat injury in B. subtilis spores heated at 250°F (121°C) for 6 seconds, and by Hashimoto et al., (1972) who identified the cortex lytic enzyme as the wet heat effected protein in B. cereus spores heated at 167°F(75°C) to 185°F(85°C) for 30 minutes. However, the above data do not necessarily relate to heat injury by "dry" heat. It is likely that the mechanisms of sublethal "dry" heat injury and wet heat injury are as different as the mechanisms of lethal injury. Unfortunately there are presently very little data available in the literature on "dry" heat injury which can be compared

with that on wet heat injury. Augustin and Pflug (1967) investigated the effect of varying the sporulation and recovery media on the heat resistance and the recovery pattern of the putrefactive anaerobe PA 3679 (a strain of Clostridium sporogenes). They found significant differences in the number of survivors of "dry" heat treatments (255-320°F/124-160°C) between complete media such as beef infusion and incomplete or synthetic media. At the lowest temperature the ratio was 4.5:1 and at the highest temperature 345:1 when spores produced on beef infusion were used. However, when spores produced on a trypticase medium were used, a constant ratio of about 12:1 was observed at all temperatures. These investigators suggested that nutritional requirements arising from genetic damage to the spore DNA could account for their results. To support this hypothesis they related the results of Zamenhof (1960), who reported that "dry" heat treatment of bacterial spores "in vacuo" resulted in mutagenic injury to the spores. This injury was manifested by alterations in the nutritional requirements of the spores. If this is the case, some similarities between wet heat and "dry" heat injury may exist, as similar results with wet heat have been reported by Campbell et al. (1965) and by Adams and Busta (1972). More recently, Chiasson and Zamenhof (1966) found that 221°F(105°C) was the minimum temperature at which a significant degree of mutation was observed in spores of B. subtilis. This effect was maximized at 239°F(115°C) and decreased with increasing temperature beyond that point. An examination of DNA "in vitro" under similar conditions gave similar results.

The only data available on high temperature "dry" heat injury are that of Bourdillion et al. (1948), who evidently did not recognize it as an indication of heat injury. They found that "wet" spores heated at temperatures between 401°F(205°C) and 457°F(236°C) showed increased survivors when the incubation time was extended from 20 hours at 37°C followed by 96 hours at room temperature. This phenomenon was neither noted at temperatures of 365°F(185°C) and below, nor in the control samples. The heat injury effect appeared to increase with temperature, and at 457°F(236°C) one hundred percent of the survivors were heat injured. It is evident that these observations need to be repeated, and that other experiments investigating other manifestations of heat injury at high temperature should subsequently be performed. These conclusions stimulated the heat injury work conducted in this investigation.

MATERIALS AND METHODS

A. General Procedures for Obtaining Time/Temperature Relationships

In order to determine the time/temperature relationships required to give uniform reductions in bacterial populations, aerosols of Bacillus subtilis var. niger spores were passed through a heated and non-heated incinerator system and the survivors were enumerated. The number surviving the heat treatment were then subtracted from the number recovered from the unheated control, providing a population reduction due to the heat treatment. The decimal reduction time (D) was then determined by dividing the time of exposure to maximum temperature by the logarithm of the population differences.

Originally, data were to be obtained at 100°F intervals between 300 and 1100°F(149 and 593°C), but more frequent samples were added as points of critical interest were identified. Also, temperatures above 800°F(427°C) were deleted from the research plan after it became evident that data could be obtained for these temperatures by extrapolating the data from temperatures of 600°F(316°C) and above.

The effects of moisture levels on the time temperature relationships were determined by varying the relative humidity of the ambient air entering the incinerator system, and then mixing the spore aerosol with the air before it entered the incinerator. This approach was selected because available data indicated that the moisture content of the heating environment is of greater impact than is the initial moisture content of the spores. Relative humidities were

selected to give the most comprehensive coverage of the total humidity range and still maintain a realistic research schedule. The relative humidity values tested were 0.5, 45 and 90 percent.

B. Preparation of Spore Crops

The initial culture of Bacillus subtilis var. niger was obtained from Mr. Martin G. Koesterer of the General Electric Company, Valley Forge, Pennsylvania, who originally obtained his culture from the U. S. Army Biological Laboratories, Ft. Detrick, Frederick, Maryland. This so called Ft. Detrick strain was used by Koesterer (1964) in his studies of dry heat resistance, and was the test organism used by the majority of investigators in the field of high temperature "dry" heat research. This culture was received early in 1966 and was maintained on Trypticase Soy Agar (BBL) slants for over three years. The spore crops used in this study were prepared in June 1969 and used during the period June 1973 to December 1974. Therefore, the average age of the spores was 4.75 $\pm$ 0.75 years at time of use.

The procedure used for the growth and preparation of the spore crops was that of Powers (1968), with a few minor exceptions. This procedure involved the growth of the organism on thin layers of Trypticase Soy Agar (BBL) in large 30 x 200 mm roll tubes. The tubes were incubated for 10 days at 32°C, at which time a microscopic examination showed approximately 95% sporulation. Twenty milliliters of sterile distilled water were added to each roll tube, which was then gently shaken by hand until all of the culture was washed

off the agar surface. The resulting suspension was then centrifuged at approximately 5100 x G for five minutes, the supernatant fluid was discarded and the packed cells were washed two more times with sterile distilled water using the same technique. Approximately 1 gram of wet spores were obtained from each roll tube using this procedure.

The wet spore preparations were then suspended in 20 ml of filter sterilized 0.1% lysozyme at pH 6.24, and gently shaken at 3°C for one hour. The suspension was then centrifuged for five minutes at approximately 5100 x G, the supernatant fluid discarded, and the spore pellet washed in distilled water three times using the same technique. This cleaned preparation examined under phase contrast microscopy showed no vegetative cells or debris, only free spores. The spores from about ten roll tubes were pooled, suspended in just enough sterile distilled water to make a pourable slurry, and then placed in 150 ml vacuum bottles (VirTis Research Equipment, Gardiner, New York). The suspensions were frozen in a thin layer by spinning the bottles in a mixture of dry ice and 90% ethyl alcohol. The frozen suspensions were then vacuum-dried to produce a light spore powder. This procedure produced spore crops which consistently contained between 5×10^8 and 5×10^9 viable spores/mg.

The spores were stored in aluminum weighing dishes in desicators over anhydrous calcium sulfate. Loss of viability over the 4 to $5\frac{1}{2}$ year period was consistently less than 70%, and averaged approximately 10%/year.

Before use, 0.1 gram portions of the dried spore preparation were gently ground in a small glass tissue grinder to produce a fine homogeneous powder. This procedure was found to produce a more easily aerosolized powder.

C. Production of Calibrated Spore Aerosols

A calibrated spore aerosol was produced from the spore powder using the spore gun and aerosol chamber shown in Figures 1 and 2. The spore gun consists of the following components: (1) a 200 psi pressure guage, (2) a DeVillbis Type IGD Air Gun, (The DeVillbis Co., Toledo, Ohio), (3) a "quick-disconnect" air hose connection, and (4) a "Unijet" nozzle TT9501 (Spraying Systems Co., Bellwood, Ill.) with a stainless steel cylinder in place of the normal filter unit. The cylinder carries the "charge" of 10mg of spore powder, and is covered on one end with a teflon membrane held in place by a rubber "O" ring. This covering allows the cylinder to be stored in a desiccator in the upright position, acting as a storage container for the spores. A flange plate around the nozzle base allows the gun to be bolted to the aerosol chamber wall.

The aerosol chamber is an all-welded aluminum box with dimensions of 15x15x10.5 inches. Opposite the side in which the spore gun is mounted are three rows of silicon rubber diaphragms (Figure 3). Ancillary to the chamber is a separate frame which has 3 series of holes opposite the diaphragms. These are used to support 10ml glass syringes which are the means of both calibrating and transporting the

Figure 1.- Exploded view of spore aerosol gun.

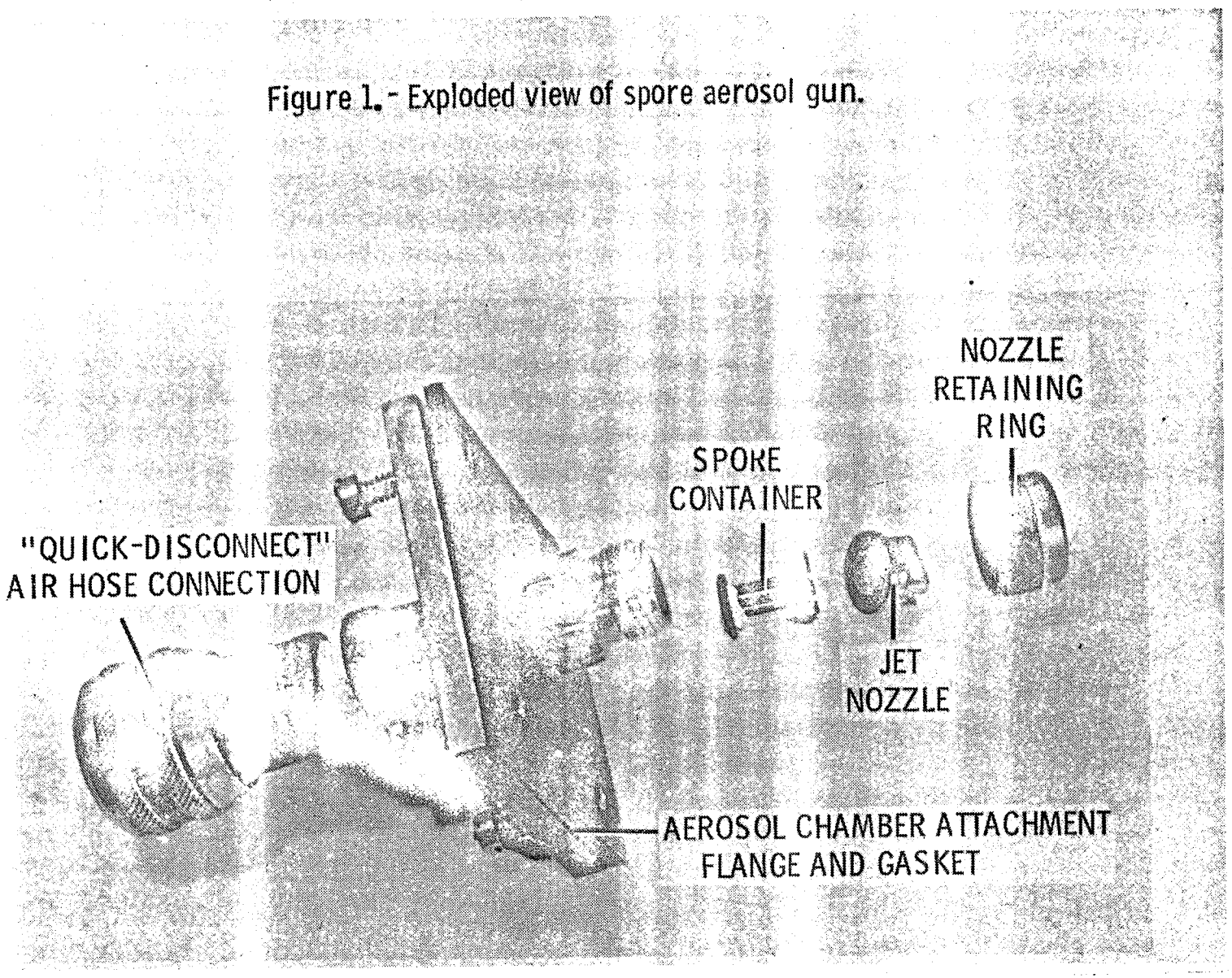


Figure 2. - Aerosol chamber with spore gun in place.

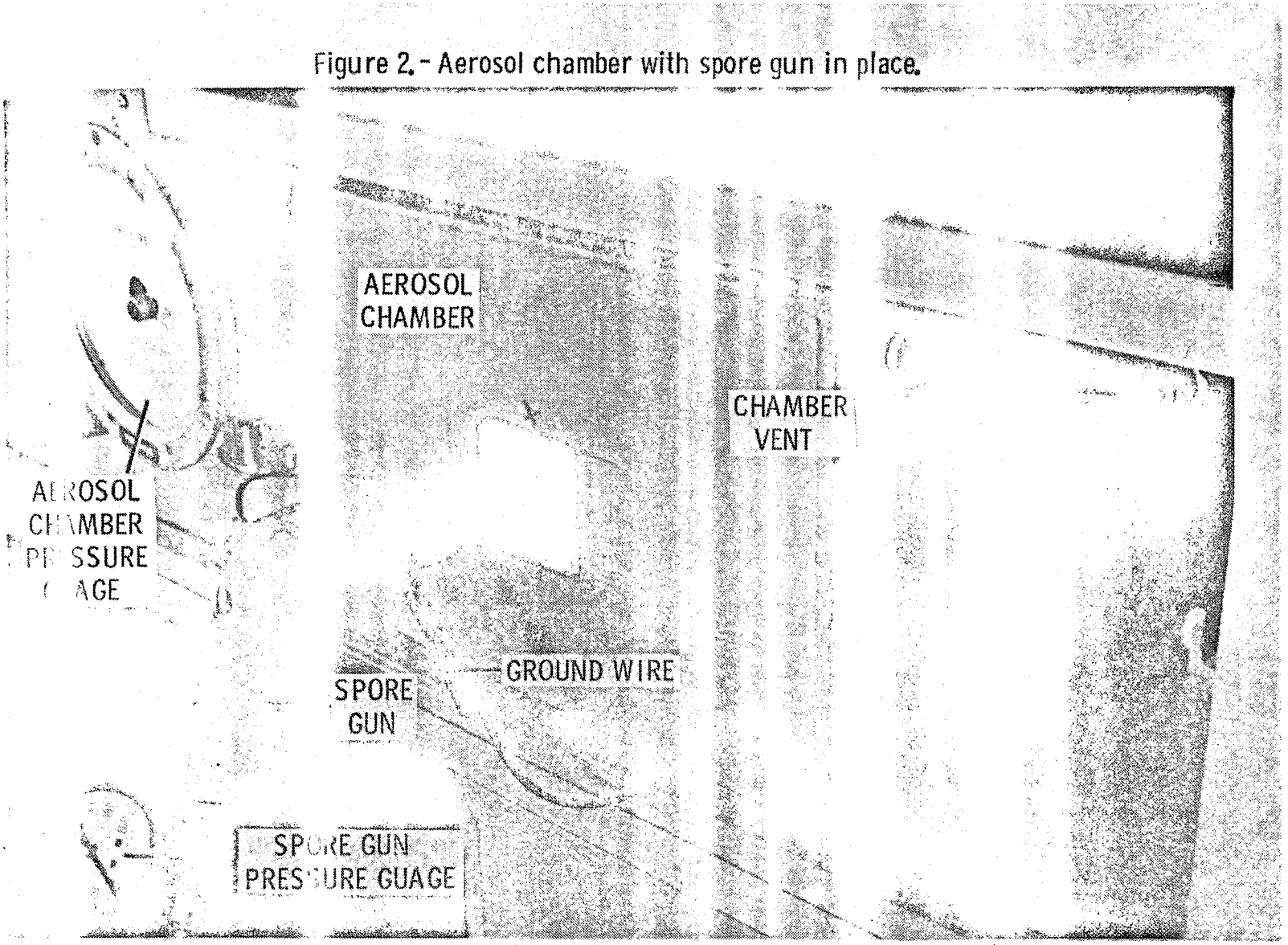
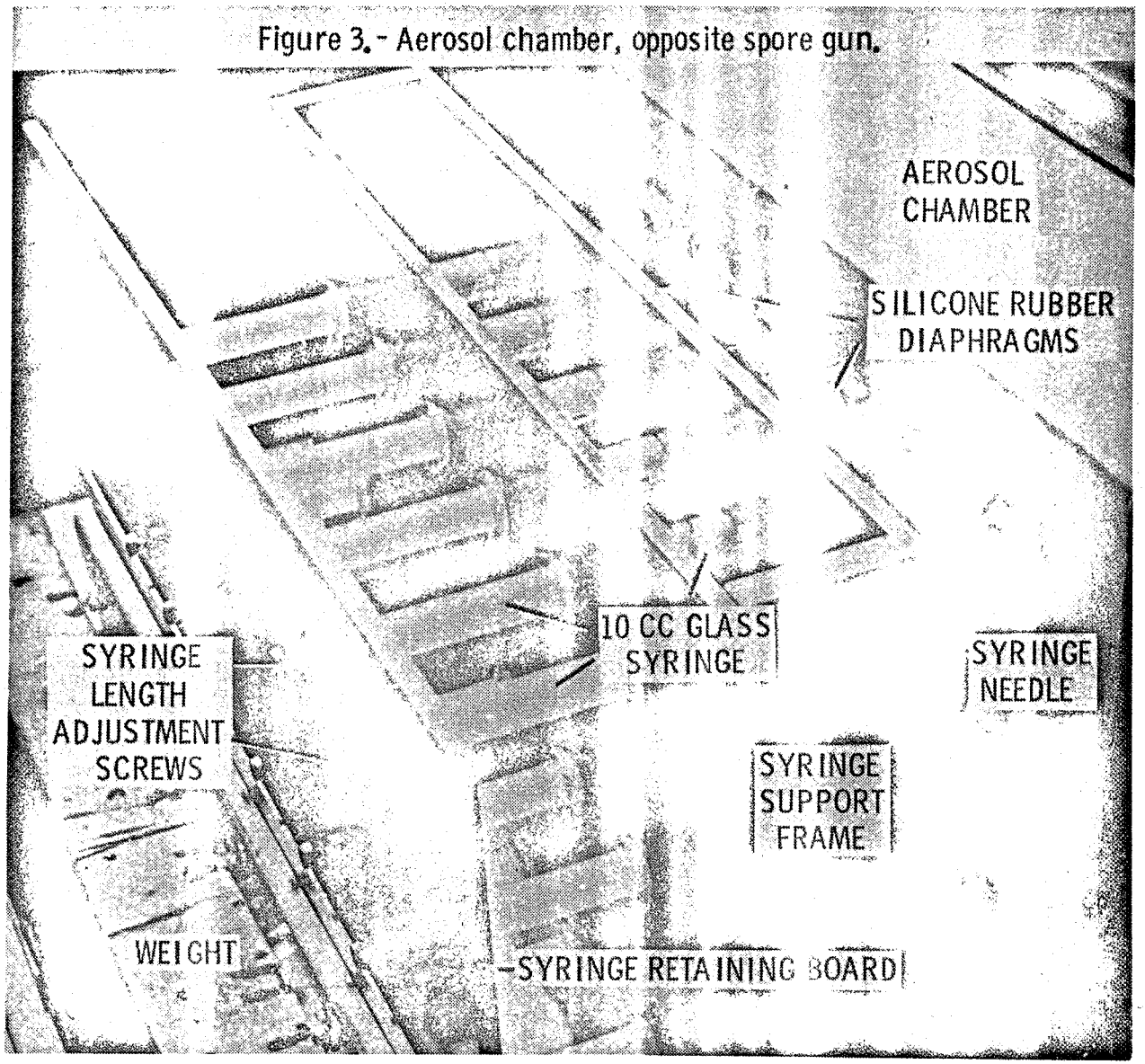


Figure 3. - Aerosol chamber, opposite spore gun.



aerosol to the test system. The syringes are of the ground glass type with 21 gauge, 2.5 inch stainless steel needles. Other components of the chamber are a vent, which includes a needle valve, glass wool depth filter, a 47mm filter holder with 0.22 micron pore size membrane filter, and a 10 psi pressure gauge, which is graduated in 0.05 psi intervals.

The aerosols were generated in the following manner: (1) The spore gun was "charged" with 10 mg of spore powder (approximately 1×10^{10} spores), attached to the chamber wall and connected to a 90 psi air line, (2) the trigger of the gun was pulled, opening a valve in the air line, (3) the incoming air flowing at 5-6 CFM ruptured the teflon diaphragm explosively, carrying the spores through the nozzle jet and into the chamber, where they were broadcast in a 90^{o} arc, (4) the air was allowed to flow until the chamber pressure reached approximately 2.0 psi, (5) the aerosol was allowed to settle for 5 minutes to allow particles greater than 5 microns in diameter to settle out (during this period of pressurization the syringe pistons were held in place by a weighted board), (6) the weighted board was pulled back just far enough that the syringes could fill up to 10 ml from the pressurized chamber, and (7) the vent on the chamber was opened very slightly to bleed the chamber pressure slowly back down to ambient pressure.

Under optimum conditions, one syringe contained about 2.5×10^{5} spores. Unfortunately only 5-10% of these spores were delivered into

the incinerator test system (see Procedure for Determination of Time/ Temperature Relationships). The average aerosol delivered to the test system has been shown by sampling with an Anderson Sampler (Anderson Samplers & Consulting Service, Provo, Utah) to contain 90% particles smaller than 2 μm, 98% particles smaller than 3.3 μm, and very few particles greater than 5 μm (Anderson, 1958).

Although it was originally believed that particles greater than 5 μm in diameter might be more likely to contain a spore which would survive the exposure to high temperature, later calculations (Carslaw and Jaeger, 1959) have shown that heat is conducted so rapidly through these particles that a particle would have to be larger than 90 μm in diameter in order to afford the interior spores any significant degree of protection.

D. The Incineration Test System

The primary test system consists of the following seven components or subsystems: (1) humidity control component, (2) relative humidity, flow rate and pressure monitoring devices, (3) the syringe injection port, (4) the incinerator, (5) a gas cooling device, (6) an impinger-type air sampler, and (7) a vacuum pump. These components are indicated in the photograph of the test system designated Figure 4, and their relative locations are indicated in the system flow diagram, Figure 5. The function and a detailed description of these components is provided in the following sections.

1. Humidity Control - Two types of humidity control devices were

Figure 4. - Experimental incinerator system

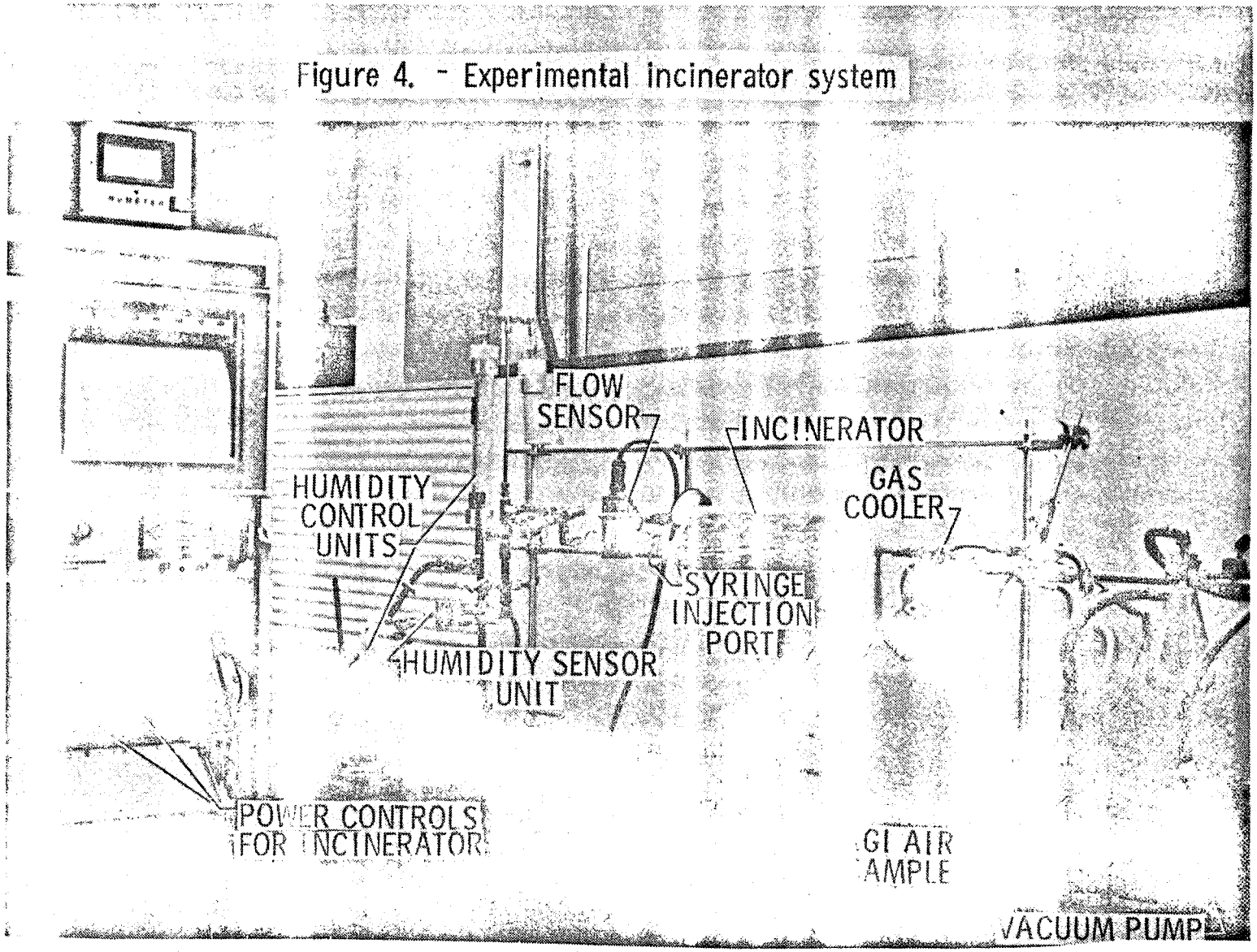
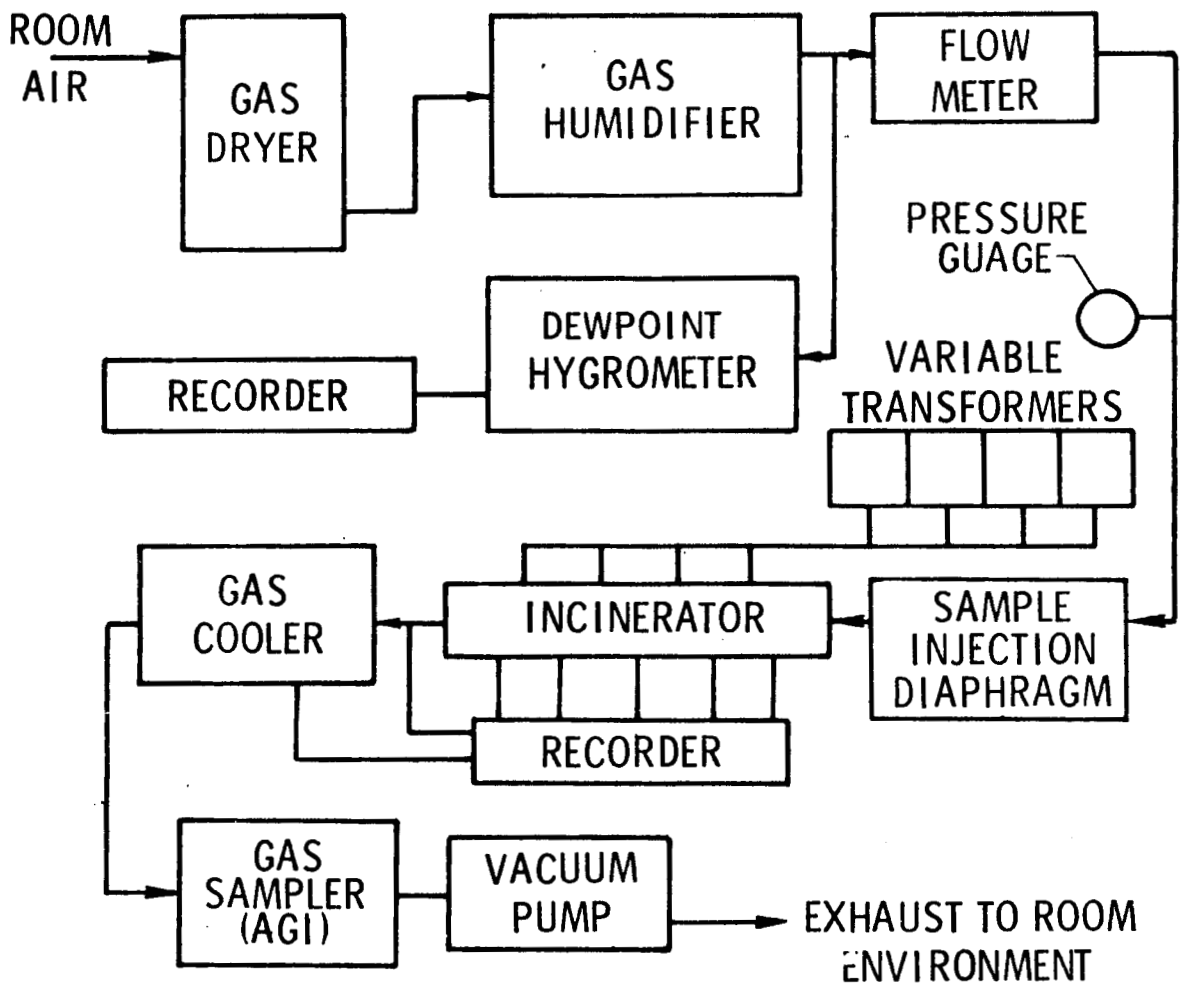


Figure 5. - Flow schematic of incinerator test system



employed in the test system, a drier and a humidifier. These were used separately and/or together depending upon the level of humidity desired. The drier consists of columns of #8 mesh anhydrous calcium sulfate which were placed in parallel so as not to restrict the air flow. The humidifiers were conventional, 700 ml, fritted disc, gas washing bottles approximately half filled with distilled water, which were also used in parallel.

2. Environmental Monitoring - Relative humidity, flow rate and pressure were monitored in that order. Relative humidity was monitored by two different systems. An El-tronics Model 101C Laboratory Hygrometer (Phys-Chemical Research Corp., New York, N.Y.) was used for the 90 percent R.H. studies, while a Cambridge Dew Point Hygrometer, Model 992 (Cambridge Systems, Inc., Newton, Mass.), was used for the 0.5 and 45 percent R.H. studies. A Cambridge Dew Point Hygrometer, Model 880, was used for a time in the latter studies when the other instrument was being repaired. The sensor for the El-tronics Hygrometer was located in the main air line, but the dew point hygrometers used a small air sample diverted from the main stream.

The flow rate was monitored so that the spore residence time in the incinerator could be determined. Since very short periods of time were involved, the flow monitoring system had to be very accurate. A Hastings Mass Flowmeter (Hastings-Raydist, Inc., Hampton, Va.) (thermal conductivity type) was used for this purpose. Both the flowmeter and the humidity monitors had continuous millivolt signal outputs which were monitored by a two-pen strip chart recorder.

The vacuum/pressure gauge was a standard bellows type which was recommended for 0-30"Hg. vacuum and 0.50 PSIG.

3. Syringe Injection Port - This component, shown in detail in Figures 6A and 6B, is the result of many designs and redesigns required to overcome a prevailing problem of static charge which retained much of the spore aerosol in the syringe needle and barrel. This final design utilizes an initial chamber filled with a saline and wetting agent (Tween 80, Fisher Scientific Co., Fair Lawn, N.J.) solution (0.9 and 0.01% respectively) which not only bleeds off the static charge already on the syringe, but also coats the needle and reduces the charge created when it passes through the second and third diaphragms. The second chamber serves to keep leakage from the first chamber from reaching the interior of the test system. The first chamber is vented to ambient pressure through a small surge tube, while the second chamber has both a drain and a vent hole. The whole unit can be easily disassembled to replace the silicone rubber diaphragms, which is required after 60 injections. The unit is grounded through the test system.

4. The Incinerator - Some history of the development of the incinerator is given in the INTRODUCTION, and therefore, will not be repeated here. The incinerator body is constructed of 0.09375" thick, Type 316 stainless steel. This design, a photograph of an early prototype is shown in Figure 7A is unique, but could easily be built by any machine shop which has experience in working with stainless

Figure 6A. - Syringe injection port.

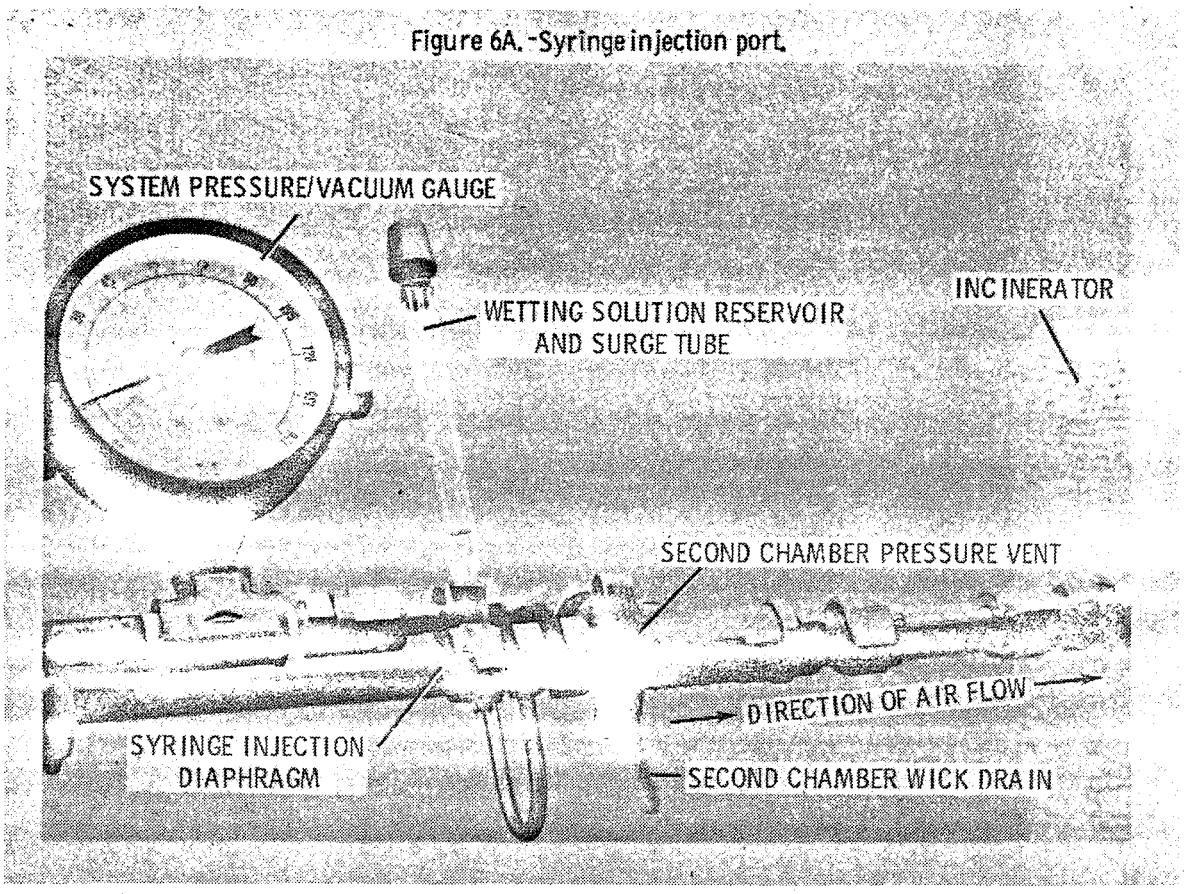
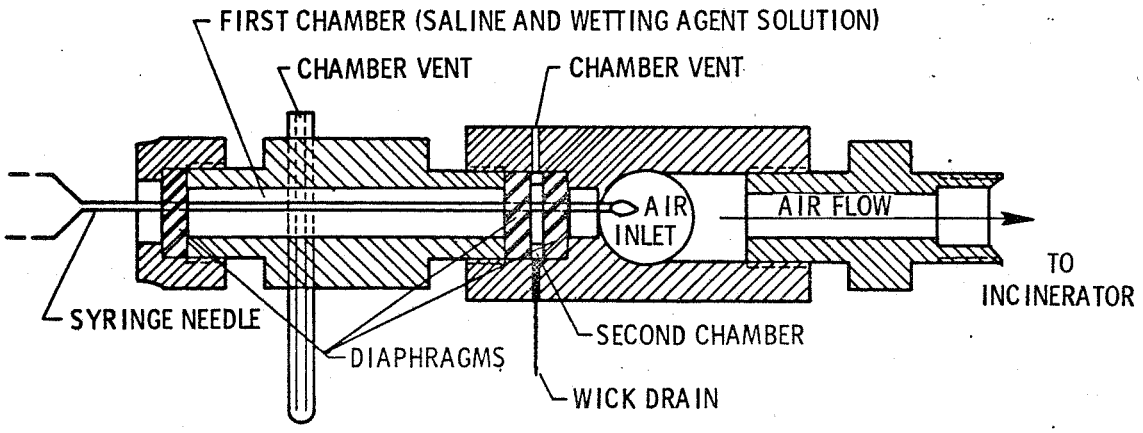


Figure 6B. - Section drawing of syringe injection port.



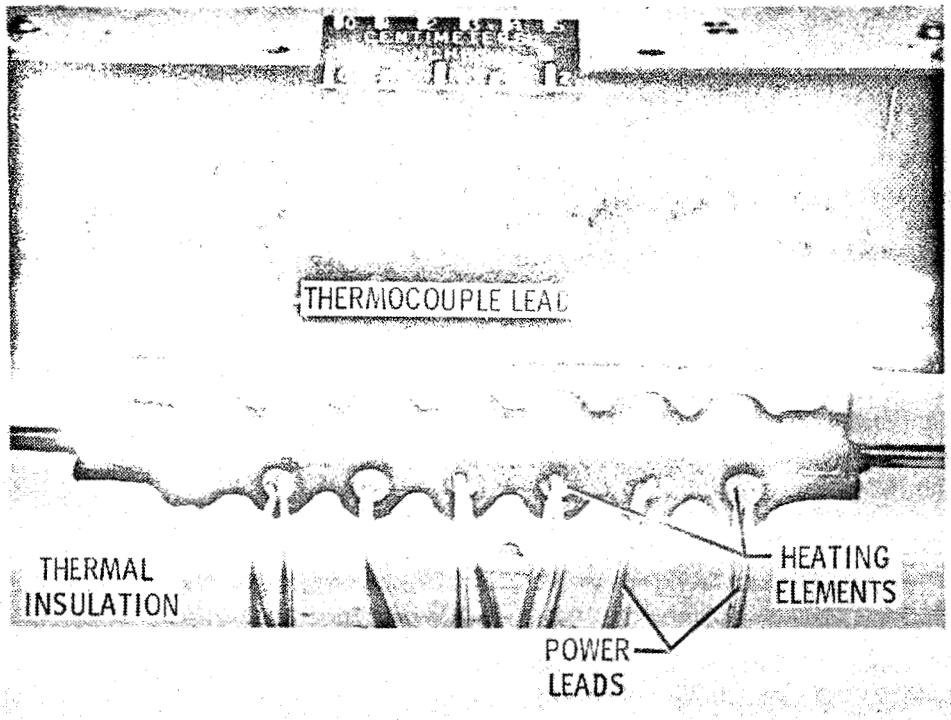
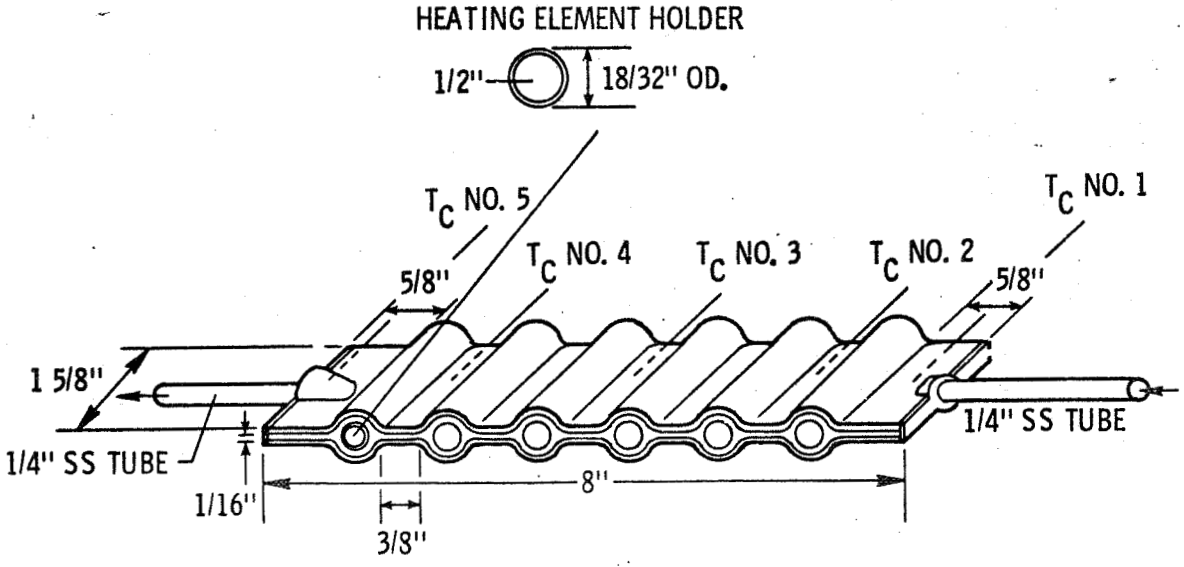


Figure 7a. - Prototype research incinerator.

Figure 7B. - Drawing of research incinerator.



steel. The primary feature of this unit is the removable heating units. These cylindrical units are 6.0" long and 0.5" in diameter (Hotwatt Cartridge Heater, Hotwatt, Inc., Danvers, Mass. #SC506), have a maximum operating temperature of 1250°F, and utilize a maximum of 100 watts at 110 volts. These units can be removed from the incinerator at any time, without violating the sterility of the incinerator or requiring any type of shut down. This is accomplished by the use of a high temperature, highly heat conductive lubricant (Fel-Pro C5-A, Antiseize Thread Compound, Fel-Pro, Inc., Skokie, Ill.), which is a copper colloid capable of withstanding temperatures up to 1800°F. The incinerator temperature is monitored by five chromel-alumel thermocouples which are sealed (swaged) to the incinerator exterior and which have exposed heads located half-way across the breadth of the unit (Figure 7B).

5. Gas Cooler - In order to rapidly cool the air from the incinerator, a simple finned cooler was constructed which consists of alternating aluminum washers and copper spacers. The 0.25" stainless steel tubing carrying the air was bent into a loop (diameter of 3"), and a 3" lobed fan is used to pass air over the cooler to remove the heat. This unit is capable of cooling 530°F air down to less than 200°F in 0.03 seconds. The entrance and exit temperatures of the cooler are monitored by chromel-alumel thermocouples similar to those used in the incinerator.

6. Air Sampler - The sampler used to recover spores after they

had passed through the incinerator and cooler was a Greenburg-Smith Impinger (Gelman Instruments Co., Ann Arbor, Mich.), sometimes called an All-Glass Impinger (AGI). This device utilizes a 2.3 mm diameter orifice to produce a strike velocity of about 100 meters/seconds on an impingement plate. The plate was continually awash in a 0.0005% (by volume) solution of Tween 80 in distilled water, a 100ml volume of which was used in each sampler. The recommended flow rate for this sampler is one CFM. The rated recovery efficiency for this type of air sampler is reported to be 99% for particles in the 0.5 to 1.0 μm range. Since the same sampler is used for both heated and unheated samples, the absolute recovery efficiency is not relevant to subsequent calculations.

7. Vacuum Pump - A standard two CFM laboratory vacuum pump was used in these studies to provide sufficient flow in the test system. Normal vacuum at the pump was 20" Hg. When reduced flows were necessary, a small manual valve in the air line was used to restrict flow.

E. Procedures Used for Experiments in Time/Temperature Relationships

A standard operational procedure was used for all experiments relating to time/temperature relationships. Only minor variations were permitted from this procedure and these variations were documented. The narrative account of this procedure is given below.

Prior to commencement of each experiment the tubing between the incinerator and the air sampler, including the gas cooler, was sterilized by passing hot air from the incinerator through the line at

maximum flow with the cooler fan off. A minimum temperature of 350°F (177°C) was maintained at thermocouple #7 for at least 30 minutes. This section of the system was not, however, sterilized between heated samples or between heated samples and the unheated controls. The air samplers were not "in-line" during this procedure, but were sterilized in a dry heat oven at 275°F(135°C) for 16-18 hours. The syringe, needles and filter units used in these experiments were sterilized with the air samplers.

About one-half hour before an experiment was commenced, a charge of spores was placed in the spore gun and discharged into the aerosol chamber. After the syringes had been filled from the chamber, and the chamber pressure bled down to ambient, the weighted restraint was removed allowing access to the 18 syringes. During the bleed-down period the voltage controls on the incinerator heating units were set to 110 V to preheat the incinerator. At this time the humidity and flow sensors were turned on and a sterile air sampler was placed in line. There was no flow in the system during this period. The gas cooler fan was turned on just before flow was started in the system.

After the maximum incinerator temperature reached 100°F above the desired test temperature, the voltage controls were set to predetermined voltages which were approximately those which would give the desired temperature once the flow was commenced. The vacuum pump was then turned on and the flow control valve was opened fully. Once temperatures in the incinerator had begun to stabilize, the

system vacuum was adjusted to 6" of mercury, the vacuum which had earlier been determined to give maximum spore delivery from the syringes. The humidity control was then adjusted to give the desired moisture level in the flowing air. Finally the temperature at the thermocouples 3, 4, and 5 was adjusted to the test temperature ($\pm 5^{o}F$) by fine control of the heater voltages. Once temperature, flow rate, moisture level and system vacuum were all controlled at the desired levels, the spores could then be injected into the system.

The spore-filled syringes were used in groups which varied with the temperature levels. A population of approximately 1×10^{4} spores/syringe was assumed. Since higher temperatures required larger numbers of spores in the challenge in order to obtain survivors, increased numbers of syringes were used with increasing temperature. For example, at $400^{o}F(204^{o}C)$ two syringes/sample were sufficient, allowing five heated samples and four controls, while at $600^{o}F(316^{o}C)$ six syringes were required per sample, which allowed for only two heated samples and one control. Syringes were selected from the 18 positions according to a predetermined scheme which distributed selections both horizontally and vertically to cancel out observed variations.

Spores from the syringes were injected into the test system using the vacuum in the system. A syringe was held so that one finger kept the piston in place while the needle was passed through the three diaphrams. Once the tip of the needle was into the air stream the piston was released and the aerosol was pulled into the incinerator. When the aerosol had been delivered, the first syringe was removed and a second

was immediately injected. This process was repeated until all syringes in a sample (2-6) had been injected. A two minute waiting period followed to allow all spores to be flushed out of the system and into the air sampler. The first sampler was then replaced (flow off) with the second and the next series of syringes were injected. This process was repeated until all heated samples had been run. The heater unit voltage controls were then turned down to 20 V, the incinerator was allowed to cool down to below 150°F(66°C), and the same process was repeated for the control samples.

Once all samples had been collected, the fluid in the air samplers was assayed to determine the number of survivors. The contents of each sampler was emptied into a 150ml flask, corked, and shaken vigorously for 30 seconds. One and 0.1 ml aliquots of the control samples were then plated in TSA to give 1 x 10^{-2} and 10^{-3} dilutions, respectively. When more than 100 survivors were expected in the heated samples, 10ml aliquots were plated in TSA to give 1 x 10^{-1} dilutions. The balance of 80ml was filtered through a sterile 0.22µm membrane filter, and the filter was placed on a TSA plate. When less than 100 survivors were expected, all 100ml of the sample was filtered. All dilutions and plating were done in duplicate.

All samples were incubated at 32°C and counted after 24 hours. Since the maximum number of countable colonies on a filter was approximately 100, the 1 x 10^{-1} dilution was counted between 10 and 300, rather than the normal 30/300.

F. Procedures for Sublethal Heat Injury Studies

At the beginning, this research program contained no heat injury experiments, simply because the phenomenon was not expected with dry heat. However, indications of heat injury were observed early in the program and procedures, based on the observations of Bourdillion et al. (1948), were developed to quantify and identify this occurrence. In addition, the variations in the recovery media was stimulated by the conclusions of Augustin and Pflug (1967) and the dual temperatures by the data of Edwards et al. (1965b).

The operational procedures for study of sublethal heat injury were superimposed on the procedures used for determining time/temperature relationships. The procedures were designed to observe any of four possible indications; a change in "D value", variations in survivor numbers with incubation time, differences between survivor numbers in samples incubated at different temperatures, and effects of recovery medium on survivor numbers. These additional procedures were all related to plating and incubation of heated and control samples. First, duplicate platings of heated and control samples were made in TSA and incubated at 45°C. In addition, duplicates were also plated in Minimal Agar (Davis, 1950) and incubated at both 32 and 45°C. Finally, when heat injury was suspected plate counts were made at 1, 2, 3, 4, and 7 days of those duplicates plated in TSA, and at 2, 4, and 7 days of those plated on Minimal Agar (MAD). This additional requirement resulted in a total of eight sub-samples being plated from the 1×10^{-1}

dilution of the heated samples, and from the 1 x 10^{-2} dilution of the controls. No filtration was used when heat injury effects were suspected.

G. Data Reduction and Analysis Procedures

1. Preliminary Data Reduction - Raw data produced during time/temperature determinations was primarily of three forms: (1) plate counts of both heated and control samples, (2) air flow rate for each heated and control samples, and (3) the temperature profile data for the incinerator and the air-cooler for each sample. The first two sets of data were subjected to a preliminary manual data reduction on an experiment by experiment basis.

The two control and two heated sample counts were first averaged to provide mean control and mean heated counts (since in cases when filters were counted, there was only one count per sample, that value was taken as the mean heated count). The respective counts were then multiplied by their dilution factors to provide total counts/sample (100ml). Finally, the logarithm of the mean heated sample count was subtracted from the logarithm of the mean control sample count. The resultant count being the decrease in spore population attributable to the effects of the heat. This term is referred to as DELTA P in future calculations.

In order to calculate the dwell or exposure time (t) for a spore in the incinerator system, the flow rate (in cm^3/sec) for each heated

sample was divided into the incinerator volume maintained at the maximum temperature ($3.68 cm^3$). Since the D value is the time required to reduce the population by 90% or the log of the population by one, then D can be computed by dividing t by DELTA P.

The log D values were then plotted against mean maximum temperature (the average of the three thermocouples which were controlled at the set temperature), and a linear regression line was determined for each relative humidity studied. This linear plot provided a means for comparing the effects of the change in relative humidity and since the D values used had not been corrected for the effects of heat-up and cool-down, this plot enabled a direct comparison with previously published data, none of which has been so corrected. Finally a Z value was calculated for each data set by taking the reciprocal of the absolute value of the regression line slope.

2. Final Data Reduction - After all data for one relative humidity had been obtained, a final, computer-run, data reduction performed four functions: (1) used curve fitting procedure to produce a continuous temperature profile to match the discrete thermocouple data, (2) calculated the lethality[1] of the temperature exposure by converting

[1] Used here to represent the lethal rate, or population reduction per unit time. In these calculations lethality was 1/D, i.e. the log of the population was reduced by 1/D per second. It is recognized that while this usage is similar to the traditional use of the term in the thermal processing of foods, it is not identical.

the temperature data to a reciprocal of D versus elapsed time plot and calculated the area under various portions of the curve, (3) corrected the total heat effect (DELTA P) by subtracting heat-up and cool-down effects to produce a population decrease which is due only to the "at temperature" heating, and (4) calculations and plotting of corrected D values from the corrected DELTA P. Each of these functions will be discussed in some detail in the following paragraphs, while the computer program (written for Control Data 6600/6500 series computer using Fortran IV language) is presented as Appendix A.

After a number of completely unsatisfactory approaches, a curve fitting program was tried which fit linear functions to the temperature data from thermocouples 1-3, and 5-7 (the heat-up and cool-down portions of the temperature curve). A general equation was used (log $T = a+b(t_1-t_0)$) where T is temperature in degrees Fahrenheit and t is elapsed time in seconds. Although this procedure resulted in a good fit, there were two drawbacks to this approach; first, it was a "least squares" approach which resulted in some of the data points not being on the curve; and second, the resultant curve was discontinuous, i.e. it did not give a smooth transition between the "at maximum temperature" portion and the limbs (the heat-up and cool-down portions) of the overall temperature curve. Thus, it was then decided to try the present approach, a "biphasic" curve, which would be more responsive to the known "multiphasic" nature of temperature data in the limbs of the curve. This approach uses two equations or functions to represent

each limb of the curve (Figure 8). The general equations were: log $T = a+b(t_1-t_0)$ for the low temperature portion of each limb (Between thermocouples 1 and 2, and between 6 and 7); and log $T = a+b(t_1-t_0)^2$, a quadratic function, for the high temperature portions of the limbs (between thermocouples 2 and 3, and between 5 and 6). This approach has been very satisfactory, giving excellent continuity and a "perfect" fit to temperature data (since each curve portion only has to fit two data points).

The effects of heat-up and cool-down of the carrier gas on a representative spore population can be seen in Figure 9. On the "actual", computer generated, survivor curve the heat-up effect is represented by that portion of the curve between thermocouples 1 and 3, and the cool-down effect by that portion between thermocouples 5 and 7. In this Figure, the curve labeled "Method #2" represents the approximation used to produce the D values presented as "uncorrected data". In order to determine the actual value of D, the proportional effects of heat-up and cool-down on the spore population had to be calculated and subtracted from the total observed effect (DELTA P). The computer program accomplished this in the following manner. First, it was necessary to calculate the lethality of each temperature. Using the mathematical relationship between T and D determined in the preliminary data reduction (linear regression equation), and the computer generated temperature/time equations, a plot of 1/D versus t was produced. An example plot of this type is presented in Figure 8.

Figure 8 - Lethality curve (1/D versus t) based on computed best fit of temperature data

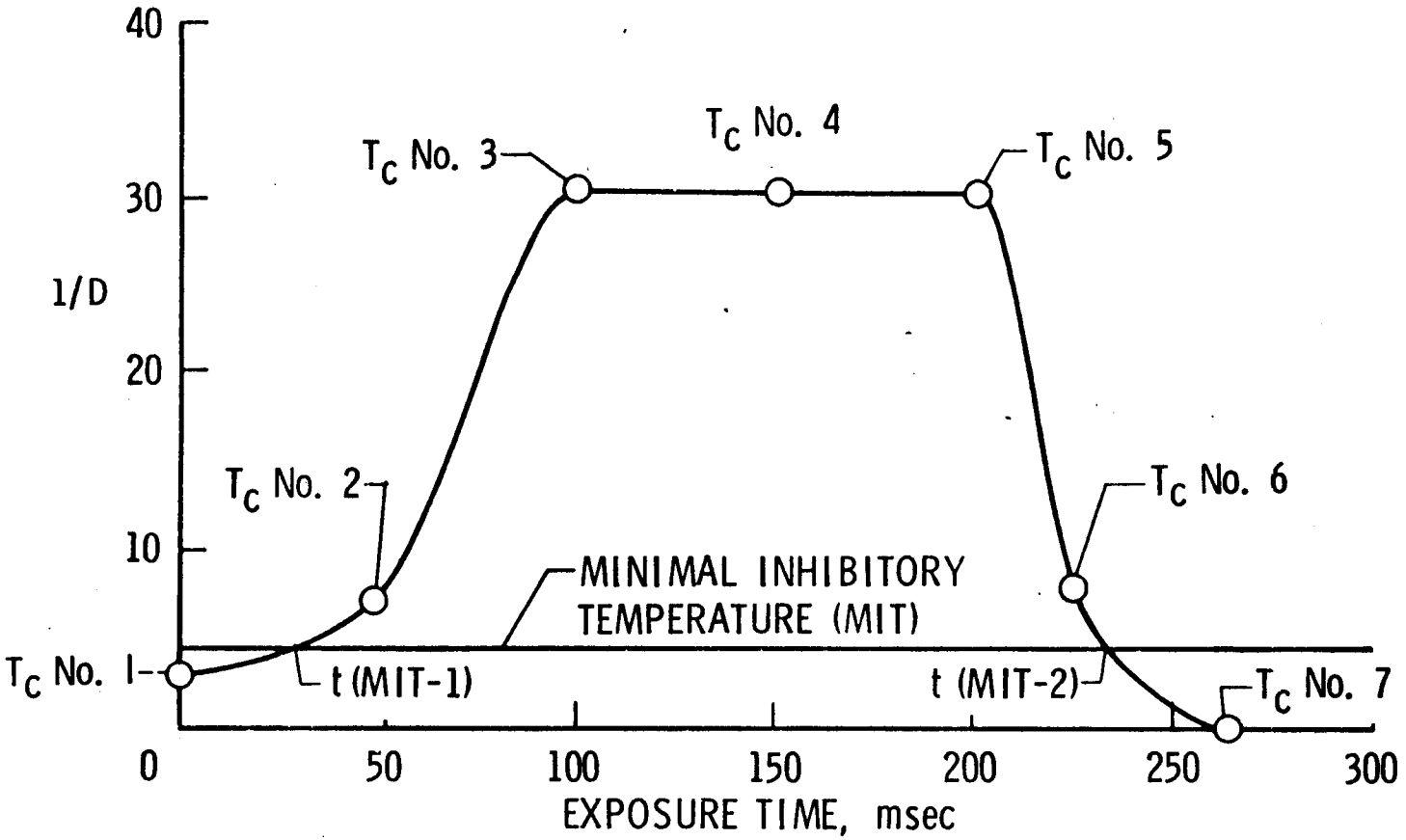
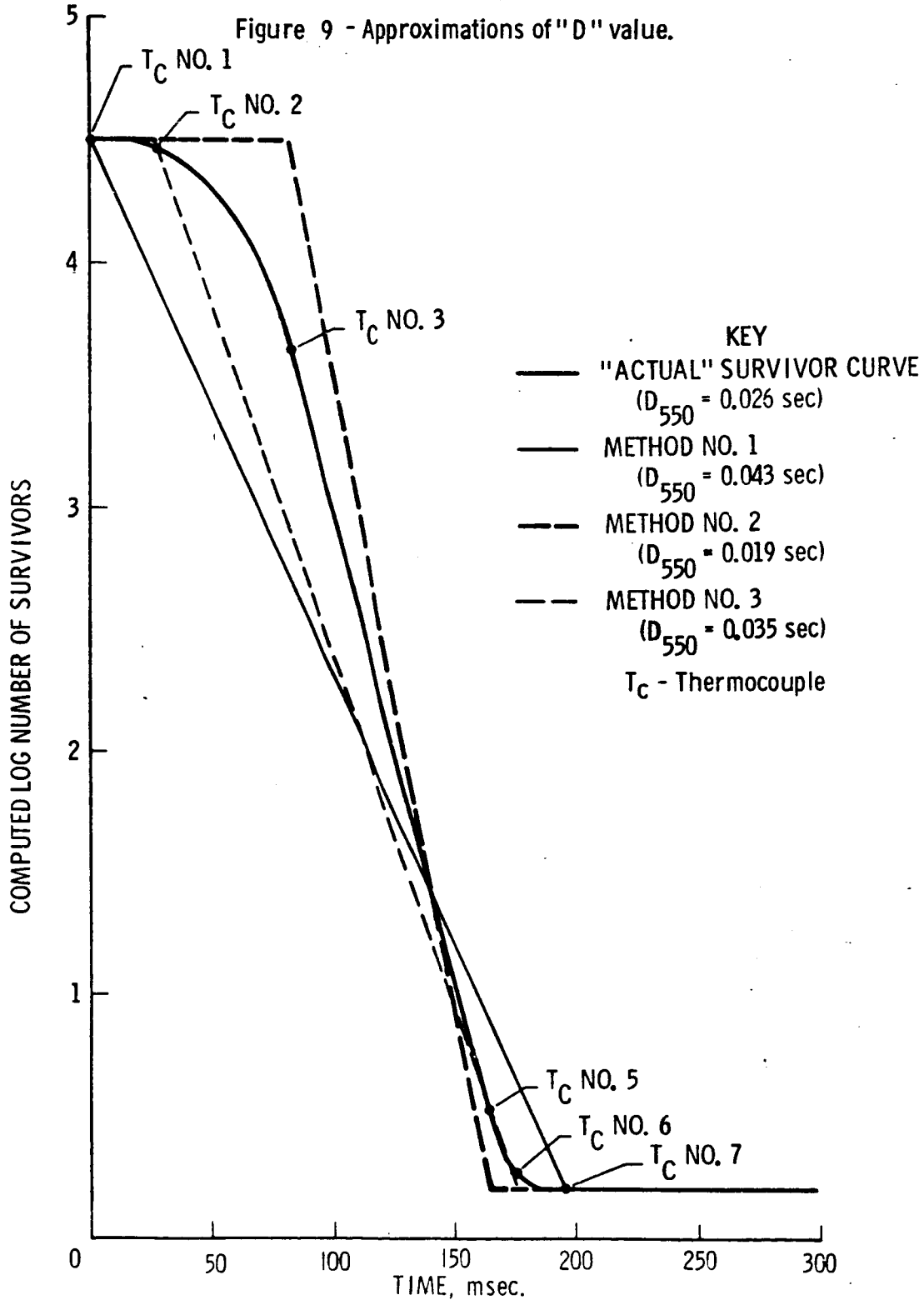


Figure 9 - Approximations of "D" value.



Determination of the areas under the heat-up and cool-down curves was by a triangle approximation technique. The bases of the triangles were the elapsed times between when the temperature attained or returned to the minimal inhibitory temperature (MIT)[1] and the time that a set-point temperature was attained or was last obtained. The heights of the triangles were the differences between the 1/D values obtained at the MIT and at the set-point temperature. The lethality curve intercept coordinates were determined using the functions obtained in the previous section of the program. For example, for the 90% R.H. data, the set-point temperatures were 400, 425, and 450°F (204, 218, and 232°C) and the MIT was determined to be 391°F(199°C). In this case, the first triangles would have heights of $1/D_{400}$-$1/D_{391}$ and bases of t(Tc#3)-t(MIT-1) and (MIT-2)-t(T_c#5) respectively. The mean DELTA P for the 400°F data set would then be corrected by subtracting the area of the triangles, and the corrected DELTA P would be utilized to calculate a corrected D_{400}(D_{400}'). The next triangles would have heights of $1/D_{425}$-$1/D_{400}$', and a D_{425}' would be calculated. This process would be continued until all corrected D values had been determined (D_{600} was used to calculate D_{450}'). The final steps of the program plot a log (1/D') versus T relationship.

[1]The minimal inhibitory temperature (MIT) was determined from an extinction plot of DELTA P, i.e. MIT was the temperature at which DELTA P should not be detectable when the heating time is 0.01 seconds, the normal exposure time for this heating system.

3. Heat-Injury Calculations - Heat injury data were reduced in three ways. First, calculations were performed as in the time/temperature studies, but using two different DELTA Ps. One DELTA P was based on the normal 24 hour plate counts as previously used, the second was based on counts at 96 hours. The uncorrected D values for each data set were calculated and the slopes of log D versus T plots were compared. Second, the effect of relative humidity was further presented by simply plotting the mean TSA plate counts of the heated samples observed at 24, 48, 72, and 96 hours. These counts were corrected for changes noted in the unheated controls. Third, the effect of recovery medium on apparent heat injury was presented by plotting the control-corrected plate counts on MAD observed at 2, 4, and 7 days. In both of the latter two computations, the data from 45 and 90% R.H. experiments was normalized to the 0.5% R.H. data.

RESULTS

A. Results of Preliminary Calculations

The raw data obtained at 0.5, 45.0, and 90.0 (± 0.5) percent relative humidity (% R.H.) are presented in Tables 2, 3, and 4. Each experiment or set of samples (indicated by date) are grouped according to the desired set temperature. Note that these temperatures were varied among relative humidities because of a desire to provide data from at least three temperatures to define each time/temperature relationship, i.e., 300, 400, 500, and 550°F at 0.5% R.H. became 400, 425, and 450°F at 90.0% R.H. because of the increased slope of the relationship at the higher R.H. Other data presented in these tables are the sample #, the actual temperature recorded at each thermocouple, and the flow rate in ft^3/min.(CFM). In addition, the results of the preliminary calculations are presented for each sample. These are the population difference due to heating, DELTA P, and D, presented as log D.

The variation of log D with temperature as a function of relative humidity is presented in Figures 10, 11, and 12. The range of log D values for each temperature is represented by an "error" bar and the number of samples taken at each temperature is presented in parentheses above the "error" bar. The linear regression equation and the correlation coefficient for each log D/temperature relationship, and the Z value for the log portion of the relationship are also given.

TABLE 2

Preliminary Data Summary - 0.5% Relative Humidity

Temperature Set-Points [1]	Experiments #	Sample #	Incinerator/Cooler Temperatures [2] 1	2	3	4	5	6	7	Flow Rates [3]	DELTA P	log D
300 (149)	8/16/73	300B	110	174	299	302	303	200	128	0.76	0.33	-0.4957
		400A	110	176	300	305	305	202	129	0.74	0.74	-0.8856
		400B	109	176	300	304	304	201	130	0.74	0.74	-0.3751
300	3/7/74	S-1	106	174	299	304	305	202	117	0.79	0.54	-0.7379
		S-2	106	175	300	304	305	200	114	0.76	0.75	-0.8638
		S-3	106	173	298	303	304	197	112	0.74	1.01	-0.9815
		S-4	106	174	299	304	305	197	112	0.75	1.21	-1.0658
		S-5	105	176	303	305	305	196	112	0.74	0.23	-0.3389
400 (204)	8/21/73	300B	118	219	401	405	405	251	144	0.76	1.16	-1.0532
		400B	119	217	400	406	407	256	148	0.78	1.20	-1.0792
		400A	118	216	398	403	404	253	146	0.77	1.35	-1.1247
500 (260)	2/11/74	S-1	128	268	500	500	498	321	160	0.81	2.81	-1.4651
		S-2	128	266	495	500	499	324	160	0.81	2.81	-1.4651
		S-3	130	271	504	505	503	321	159	0.80	2.51	-1.4107
		S-4	128	266	495	501	500	321	159	0.83	2.81	-1.4757
		S-6	128	266	496	498	497	320	162	0.81	2.34	-0.3856
550 (288)	4/16/74	S-1	133	292	552	551	549	370	189	0.96	4.45	-1.7340
		S-2	132	289	548	549	545	366	185	0.95	3.75	-1.6551
		S-3	131	289	548	550	547	372	190	0.95	1.89	-1.3386
		S-4	133	292	550	550	549	370	187	0.94	2.34	-1.4771

[1] Degrees F (Degrees C) [2] At Thermocouple #, Degrees F. [3] In CFM.

TABLE 2 (Cont'd.)

Preliminary Data Summary - 0.5% Relative Humidity

Temperature Set-Points[1]	Experiment #	Sample #	Incinerator/Cooler Temperatures[2] 1	2	3	4	5	6	7	Flow Rate[3]	DELTA P	log D
550	4/25/74	S-5	131	291	552	552	550	364	181	0.90	3.68	-1.6515
(288)		S-6	129	288	552	553	552	376	192	1.00	4.58	-1.7510
		S-7	129	289	551	554	550	370	186	0.95	3.88	-1.6790
		S-8	129	288	548	549	547	370	187	0.96	4.66	-1.7540
600	3/20/74	S-1	139	311	598	603	603	408	209	0.90	2.64	-1.4838
(316)		S-2	140	313	599	599	597	403	208	0.90	3.75	-1.6362
		S-3	141	315	601	603	601	400	205	0.90	4.05	-1.6696
		S-4	140	313	599	604	603	404	210	0.96	3.45	-1.6280
	3/26/74	S-4	136	312	601	605	603	398	204	1.00	3.10	-1.5993
		S-5	138	314	601	604	600	387	194	0.94	3.47	-1.6214
		S-6	137	313	602	599	597	389	196	0.99	4.25	-1.7319
	11/26/74	S-3	151	353	601	600	600	382	194	0.93	3.70	-1.6445
		S-4	151	353	603	601	600	384	197	0.96	4.10	-1.7026
700	4/5/74	S-1	154	364	698	703	700	451	224	0.87	3.82	-1.6295
(371)		S-2	159	367	698	704	701	450	222	0.85	3.90	-1.6284
		S-3	157	366	700	701	700	455	220	0.89	3.81	-1.6382
		S-4	152	365	703	703	702	456	217	0.90	3.88	-1.6510
		S-5	172	411	703	701	699	436	213	0.91	3.98	-1.6668
		S-6	171	406	696	700	699	447	221	0.95	3.98	-1.6855
800	4/5/74	S-5	162	416	800	804	801	506	229	0.87	3.82	-1.6668
(427)		S-6	164	418	801	803	803	509	233	0.87	3.85	-1.6855
	11/19/74	S-1	180	461	798	800	801	496	234	0.90	4.25	-1.6905
		S-2	181	462	798	800	800	496	233	0.90	4.25	-1.6905

[1]Degrees F (Degrees C). [2]At Thermocouples #, Degrees F. [3]In CFM.

TABLE 3

Preliminary Data Summary - Relative Humidity = 45%

Temperature Set-Points[1]	Experiment #	Sample #	Incinerator/Cooler Temperatures[2] 1	2	3	4	5	6	7	Flow Rate[3]	DELTA P	log D
400	8/16/74	S-1	116	219	400	401	402	276	156	0.99	0.430	-0.7370
(204)		S-2	115	216	495	403	404	276	156	0.98	0.860	-1.0336
	5/2/74	S-3	112	214	396	401	401	279	151	0.98	0.460	-0.7619
		S-4	113	217	400	404	403	278	151	0.97	0.546	-0.8319
		S-5	114	218	400	405	404	275	148	0.90	0.640	-0.8683
		S-6	113	215	400	401	399	281	156	1.02	0.658	-0.9347
450	9/12/74	S-1	125	244	450	453	453	303	164	0.96	1.440	-1.2474
(232)		S-2	125	246	450	450	449	299	161	0.93	1.320	-1.1969
500	5/9/74	S-4	131	266	498	499	496	340	174	0.95	3.816	-1.6672
(260)	5/15/74	S-1	131	269	498	501	498	395	262	0.90	3.643	-1.6236
		S-2	130	269	500	500	498	340	180	0.94	3.041	-1.5640
		S-3	130	269	499	501	500	334	177	0.91	3.041	-1.5500
		S-6	126	268	504	505	504	343	184	0.94	3.500	-1.6251
	5/28/74	S-2	124	265	499	501	500	338	178	0.97	3.463	-1.6341
		S-3	126	269	503	505	504	336	176	0.94	3.639	-1.6420
	7/23/74	S-3	128	268	500	504	501	333	174	0.93	3.747	-1.6501
500 (260)	9/7/74	S-3	139	300	501	500	500	327	171	0.94	3.570	-1.6337
550	11/11/74	S-3	150	331	553	547	545	354	187	0.915	3.760	-1.6445
(288)		S-4	150	330	552	549	549	360	190	0.95	3.340	-1.6094
600	9/30/74	S-1	157	335	599	597	598	387	202	0.95	3.350	-1.6107
(316)		S-2	160	361	605	599	599	384	200	0.915	3.050	-1.5536
	11/14/74	S-3	156	355	602	599	600	381	192	0.91	4.230	-1.6933

[1] Degrees F (Degrees C). [2] At Thermocouple #, Degrees F. [3] In CFM.

TABLE 3 (Cont'd.)

Preliminary Data Summary - Relative Humidity = 45%

Temperature Set-Points[1]	Experiment #	Sample #	Incinerator/Cooler Temperatures[2] 1	2	3	4	5	6	7	Flow Rate[3]	DELTA P	log D
700	10/9/74	S-2	175	413	704	700	696	442	218	0.90	3.800	-1.6419
(371)		S-6	174	411	700	697	696	442	218	0.90	4.030	-1.6675
	5/28/74	S-6	152	365	700	700	699	462	231	0.98	3.440	-1.6357

[1] Degrees F(Degrees C) [2] At Thermocouple #, Degrees F. [3] In CFM.

TABLE 4

Preliminary Data Summary - 90% Relative Humidity

Temperature Set-Points[1]	Experiment #	Sample #	Incinerator/Cooler Temperatures[2] 1	2	3	4	5	6	7	Flow Rate[3]	DELTA P	log D
400	1/20/75	S-2	120	242	400	401	402	270	154	0.95	0.414	-0.7026
(204)		S-4	120	243	401	402	404	271	153	0.95	0.620	-0.8794
425	12/11/74	S-1	127	257	421	424	426	280	154	0.895	1.34	-1.1868
(218)		S-2	127	257	421	422	424	279	153	0.900	1.44	-1.2205
		S-3	127	258	424	425	426	279	152	0.885	1.62	-1.2644
		S-4	126	257	422	422	422	278	151	0.890	1.62	-1.2668
		S-5	127	260	426	426	426	277	150	0.870	1.49	-1.2206
	1/13/75	S-1	124	255	422	422	424	279	156	0.915	1.30	-1.1833
		S-2	124	257	426	428	429	284	157	0.910	0.88	-1.0114
		S-3	125	258	428	428	430	281	155	0.905	1.52	-1.2464
		S-4	123	256	426	428	429	284	158	0.925	1.02	-1.0826
		S-5	123	255	422	421	422	277	154	0.900	1.08	-1.0956
450	12/17/74	S-2	130	270	448	450	452	296	161	0.910	2.61	-1.4836
(232)	12/19/74	S-3	126	270	451	451	452	295	160	0.915	2.52	-1.4707
		S-4	124	267	448	447	447	292	157	0.920	2.31	-1.4353
600	12/23/74	S-5	156	351	597	597	594	381	196	0.885	4.08	-1.6655
(316)		S-6	155	353	602	602	602	388	201	0.925	3.48	-1.6156
800	1/2/75	S-5	183	458	800	803	804	503	241	0.890	3.85	-1.6428
(427)		S-6	183	461	802	801	800	504	241	0.910	4.24	-1.6943

[1]Degrees F (Degrees C) [2]At Thermocouple #, Degrees F. [3]In CFM.

Figure 10. **0.5 PERCENT RELATIVE HUMIDITY**

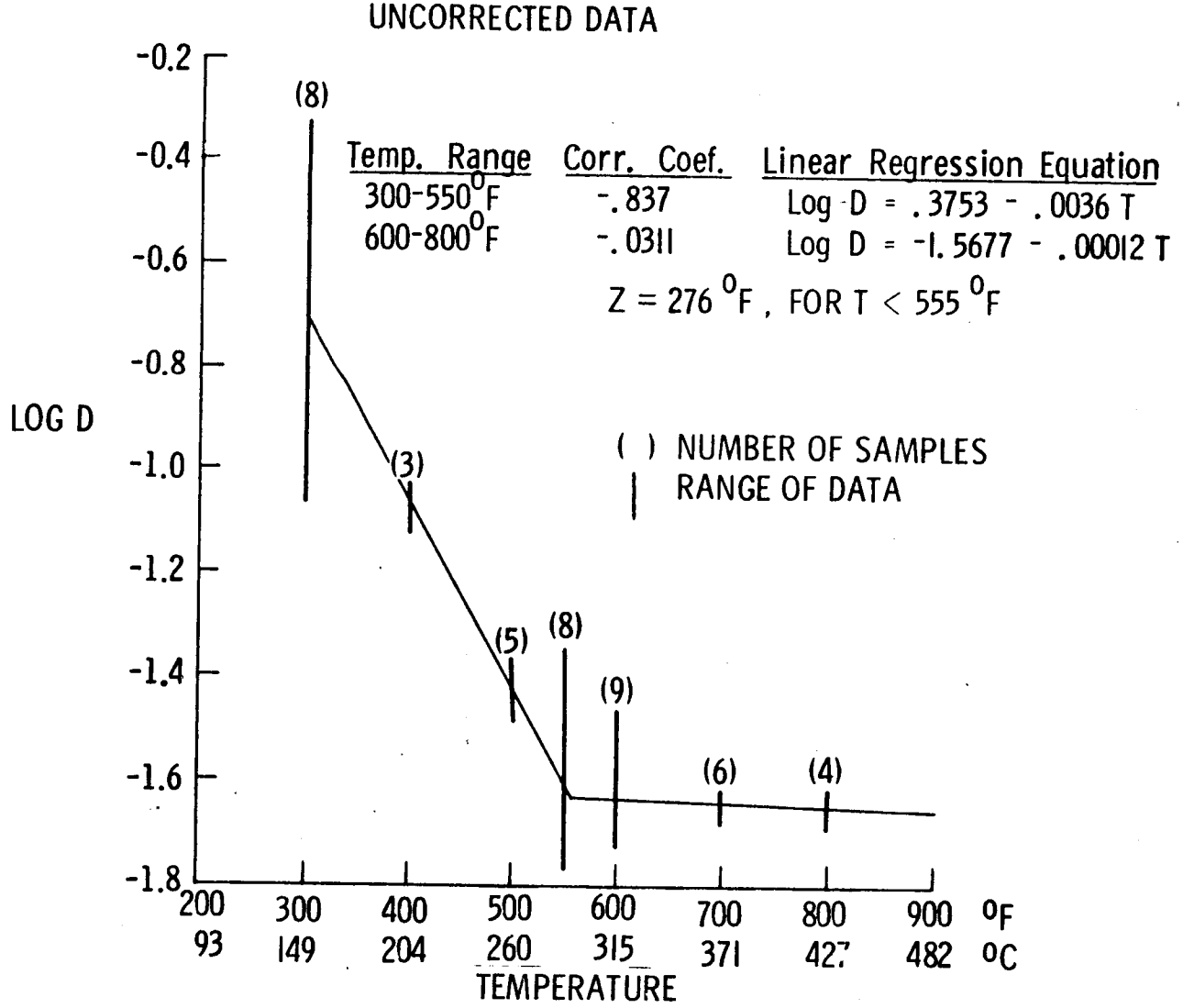


Figure II. **45.0 PERCENT RELATIVE HUMIDITY**

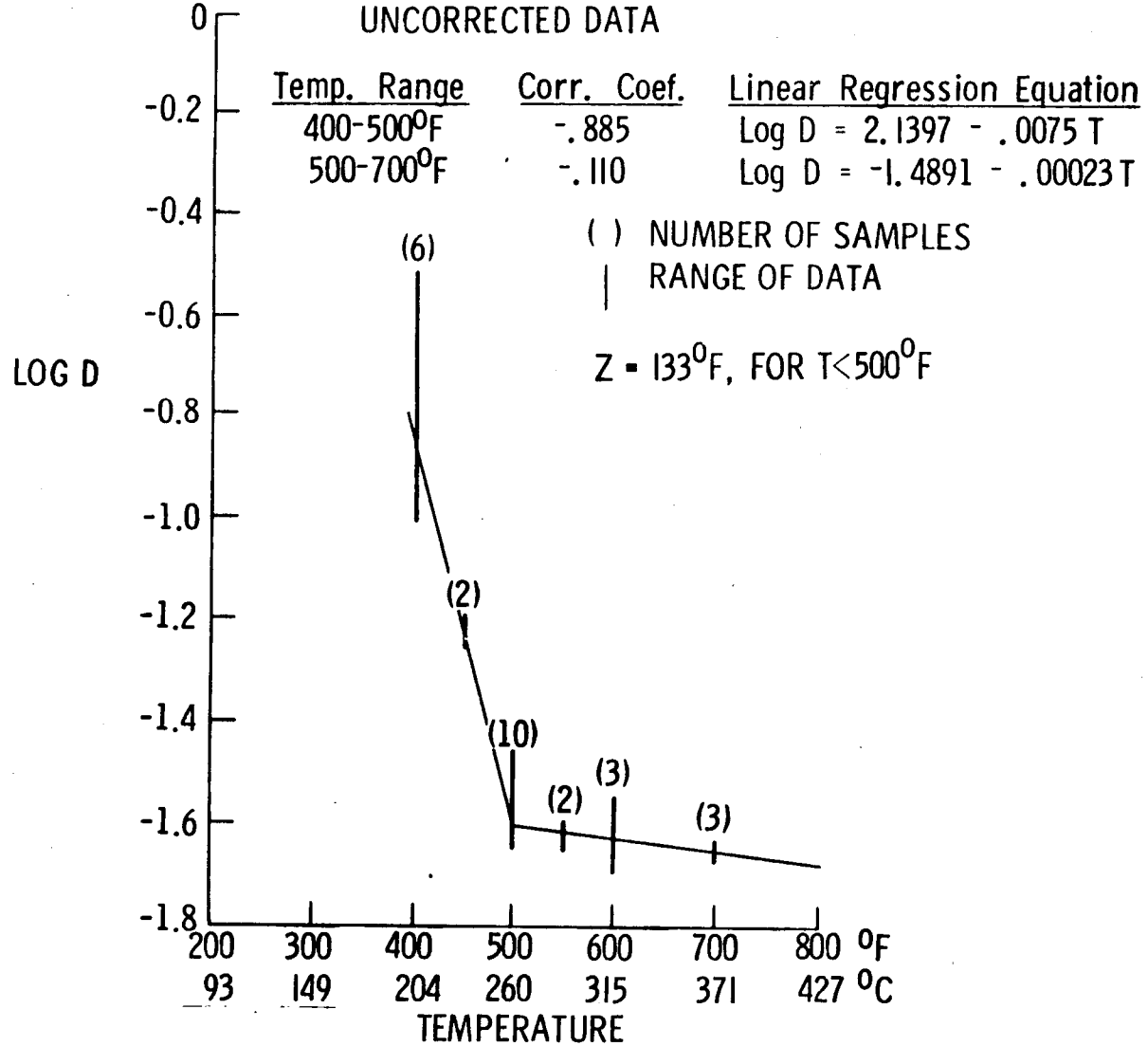
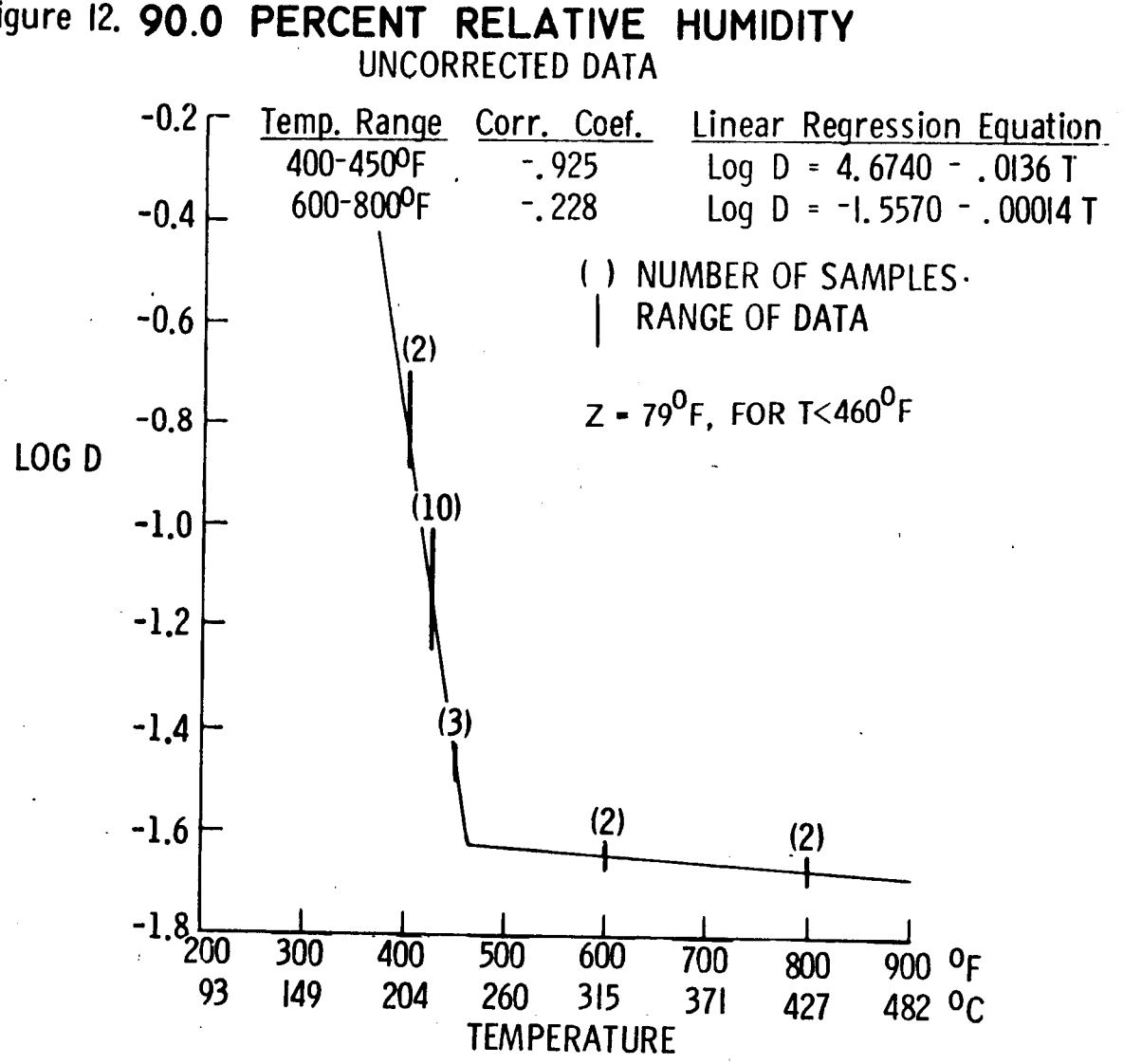


Figure 12. **90.0 PERCENT RELATIVE HUMIDITY**



A summary of the effects of relative humidity on the log D/ temperature relationship is presented in Figure 13. Here only the log portion of the relationships are represented. The Z value for each linear regression line is also given. The inverse relationship between relative humidity and Z value noted in this figure is more graphically presented in Figure 14, where the log of Z is plotted against percent relative humidity measured at 70°F(21°C).

B. Results of Final Data Reduction

Figure 15 presents a comparison of the log D/temperature relationships produced by the two methods of data reduction. The upper line shows the relationship produced when the DELTA P has been corrected for the lethal effects of heat-up and cool-down, while the lower line is based on uncorrected DELTA P's. The linear regression equation and the Z value for each relationship is also presented. The 0.5% R.H. data were used for this example. The correction of the data from the other relative humidities produced similar plots.

If the reciprocal of the corrected D, or the lethality of a temperature is plotted against the electric power (in watts) required by the incinerator system in order to heat the flowing air to that temperature, a relationship similar to that seen in Figure 16 is obtained. In this case, the data produced at 45.% R.H. were used because this is the relative humidity closest to that found in most applications situation.

Figure 13.

SUMMARY OF RELATIVE HUMIDITY EFFECTS

UNCORRECTED DATA

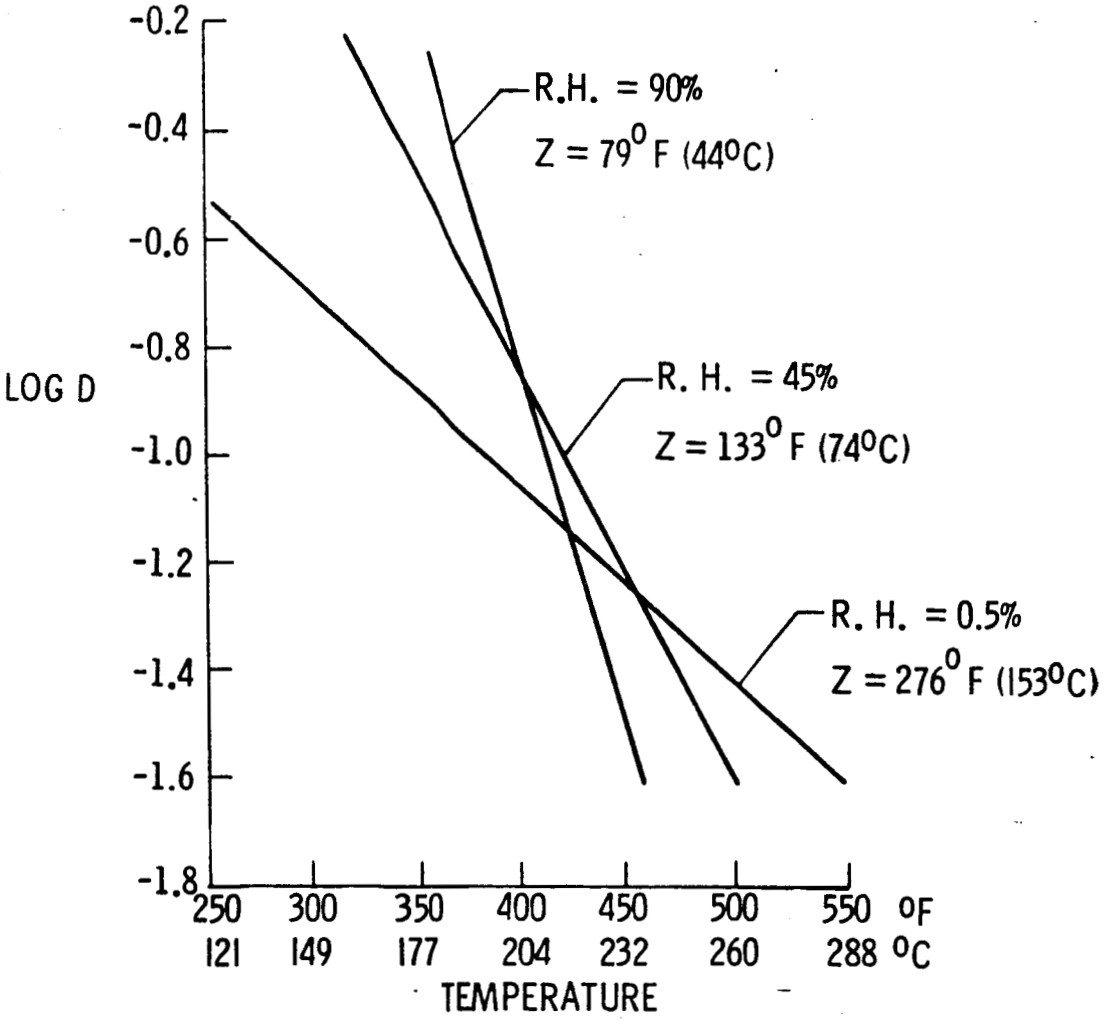


Figure 14.

RELATIONSHIP OF LOG Z TO RELATIVE HUMIDITY

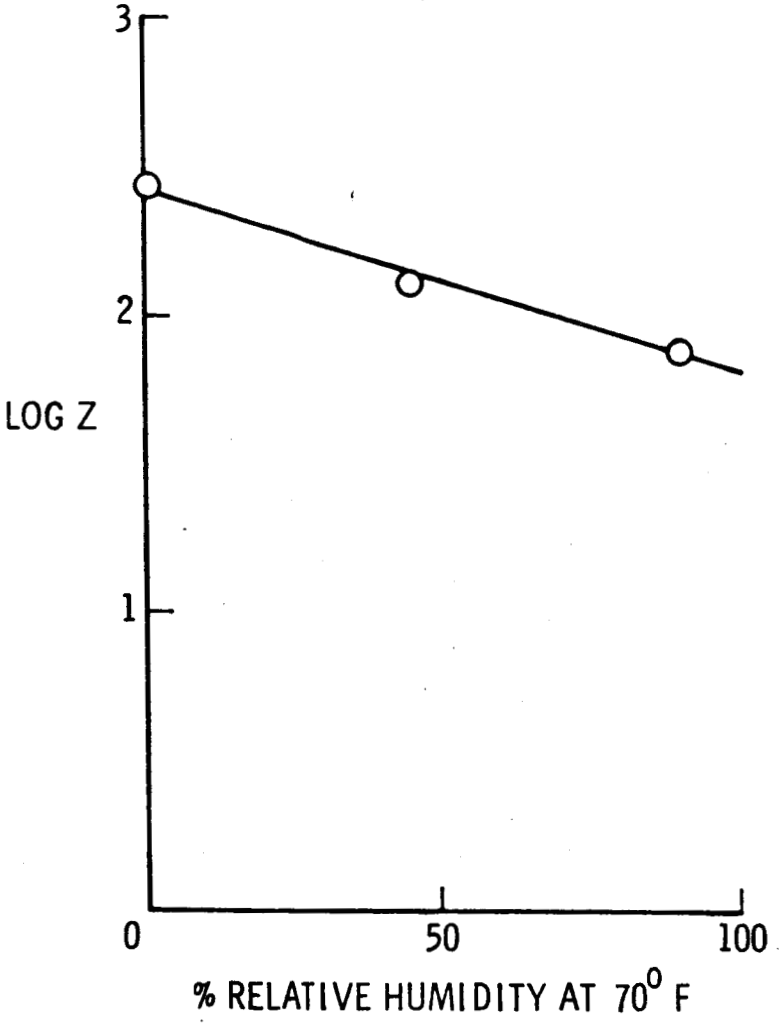


Figure 15 - Effect of correcting D for heat-up and cool-down (0.5 % relative humidity)

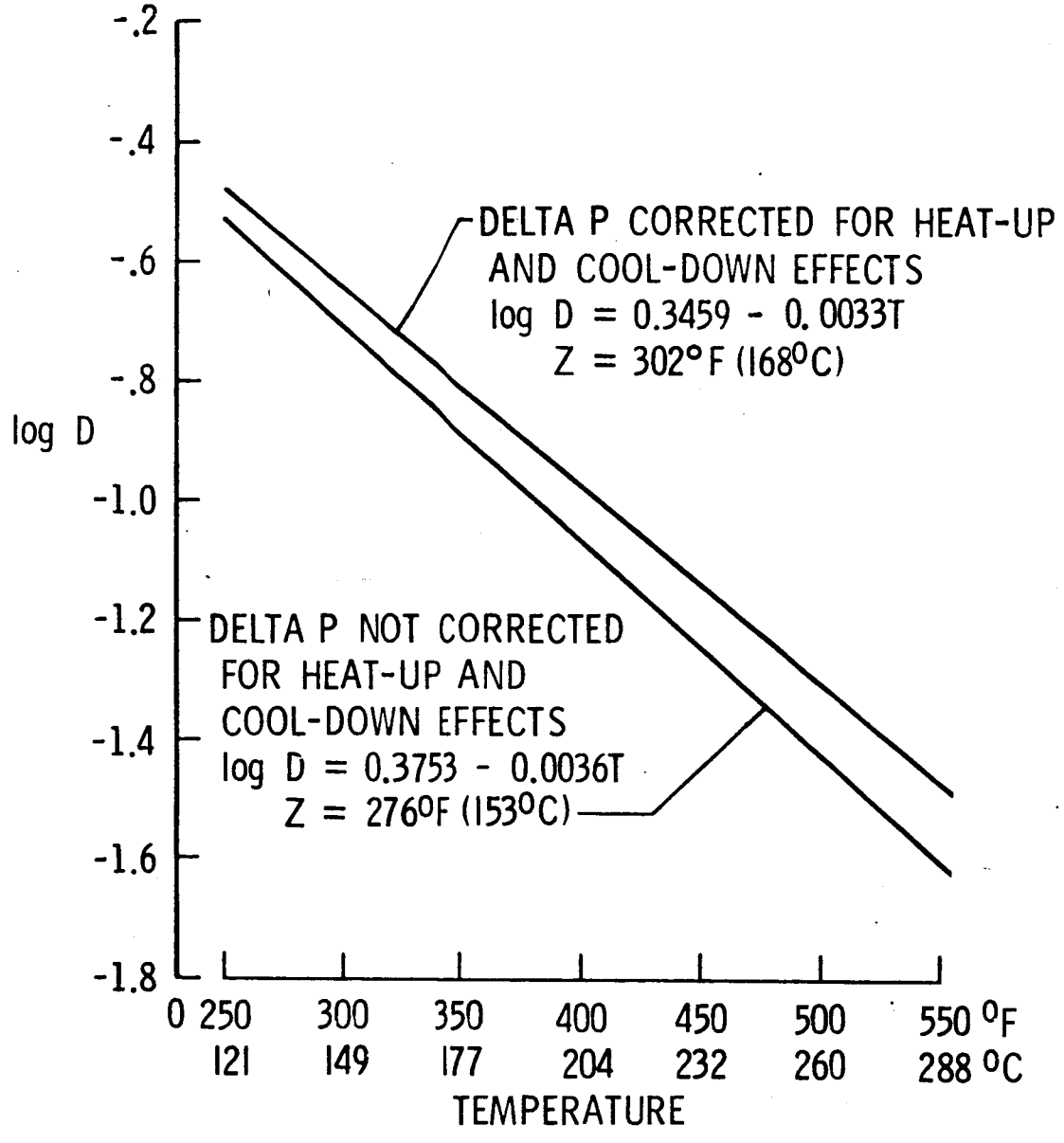
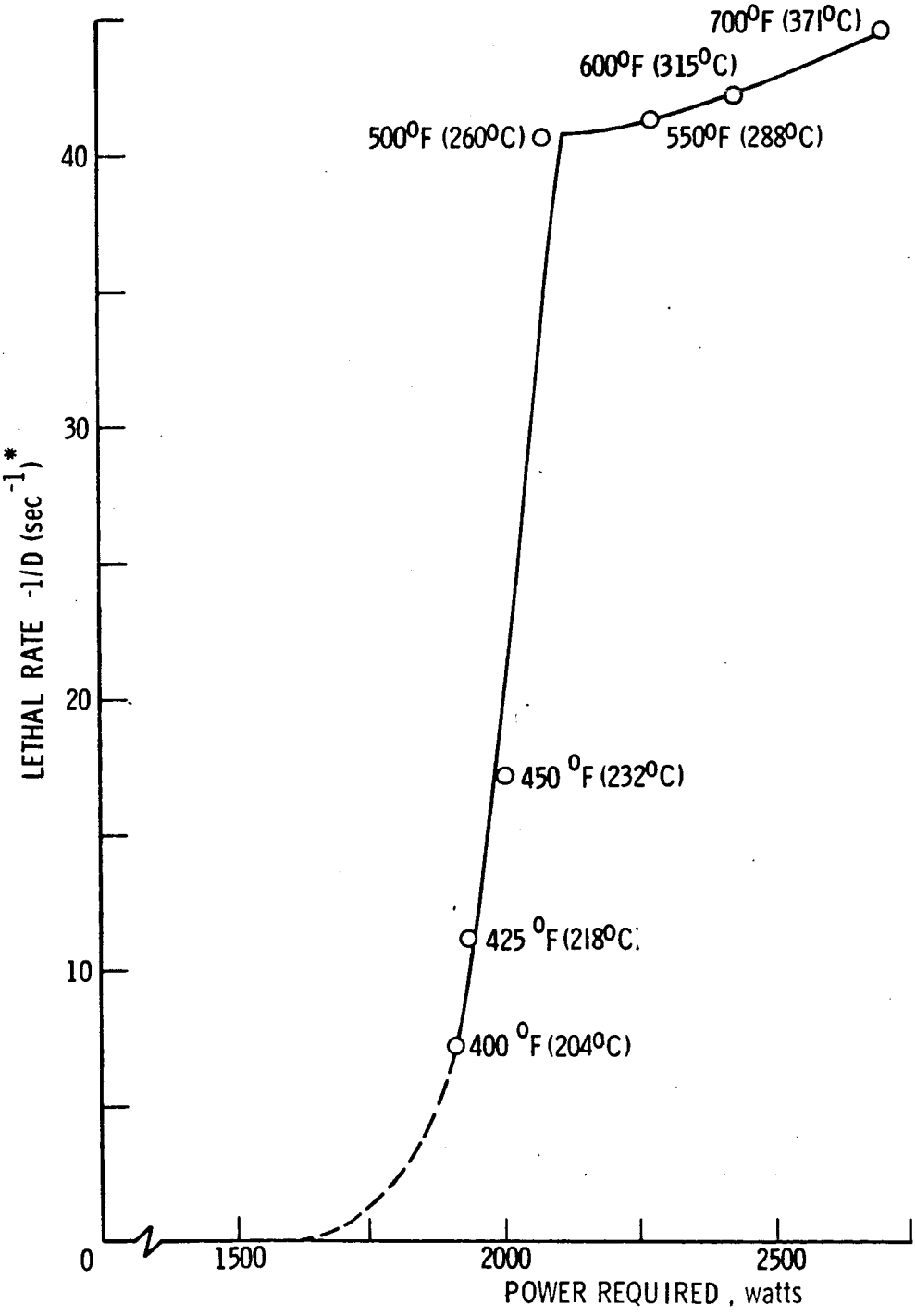


Figure 16. - Correlation between power required and lethality.



* @ 45 % R.H. (UNCORRECTED DATA)

C. Heat Injury Experiments

The primary effect noted in the heat injury experiments was the lag in germination or growth demonstrated by the heated spores on both the complete (TSA) and minimal (MAD) media. This effect is easily observed in Figures 17 and 18 which present both the population reductions obtained at the three relative humidities at 425°F(218°C) and the recovery effect observed over a period of three days incubation at 32°C. The initial populations and the plate counts for the three relative humidities and the controls have been made directly comparable by normalizing the survivors of the 90.0 and 45.% R.H. heat process enumerated at 24 hours to those enumerated at 24 hours after heating at 0.5% R.H. The increase in counts observed with the control samples during the incubation periods, although slight, have also been normalized and presented. Note that two types of survivor plots are presented in Figures 17 and 18; first, a plot of the population reductions observed at the various relative humidities when 24 hour (on TSA) and 96 hour (on MAD) incubation counts were used to enumerate survivors (called "Apparent Survivor Curves"), and second, plots of the population reductions observed at the same relative humidities when four day (on TSA) and seven day (on MAD) incubation counts were used to enumerate survivors (called "Actual Survivor Curves"). The effects noted at an incubation temperature of 45°C on TSA were virtually identical to those presented in Figure 17. However, the survivor and control counts at that temperature on MAD were always zero.

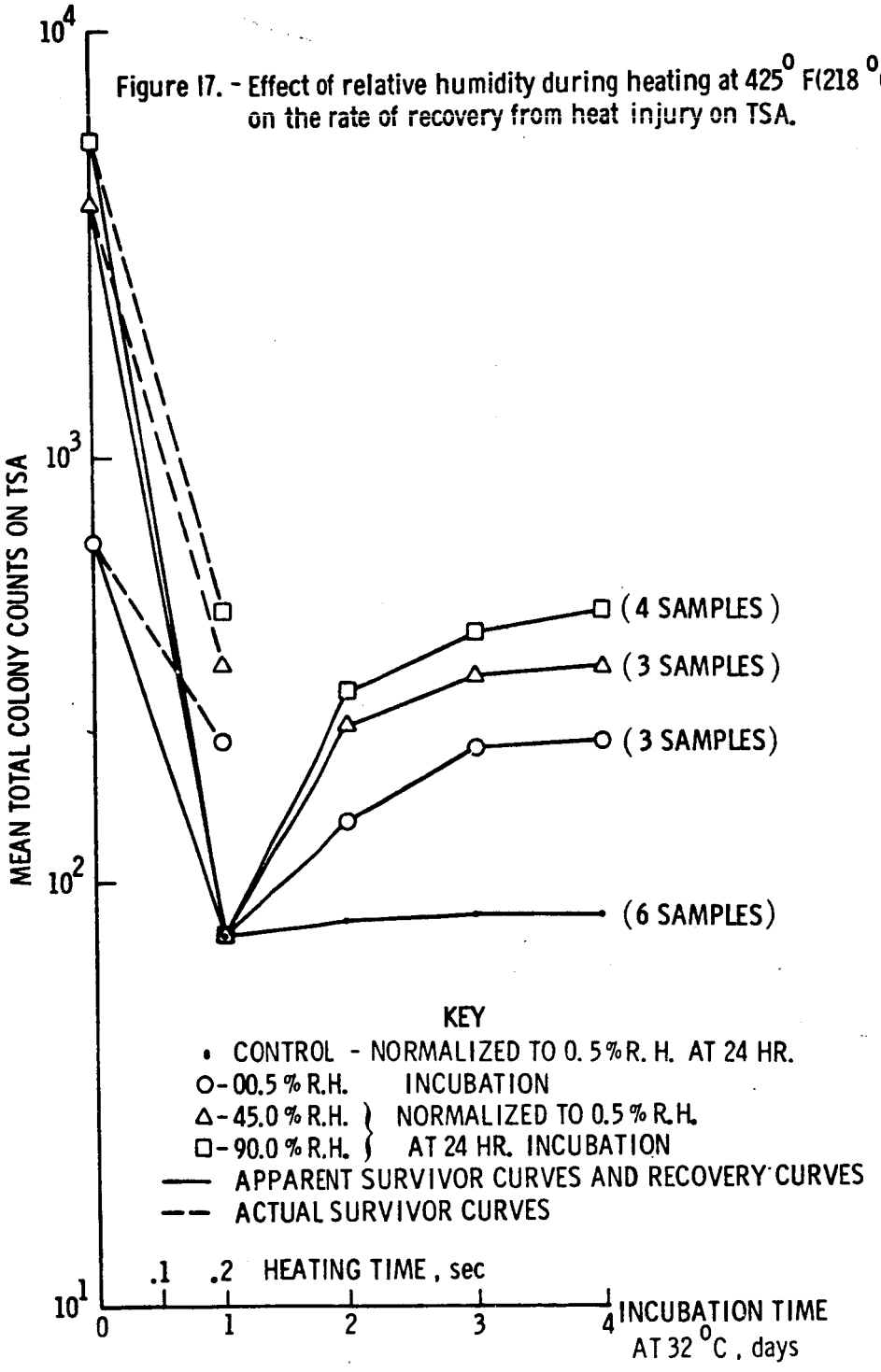
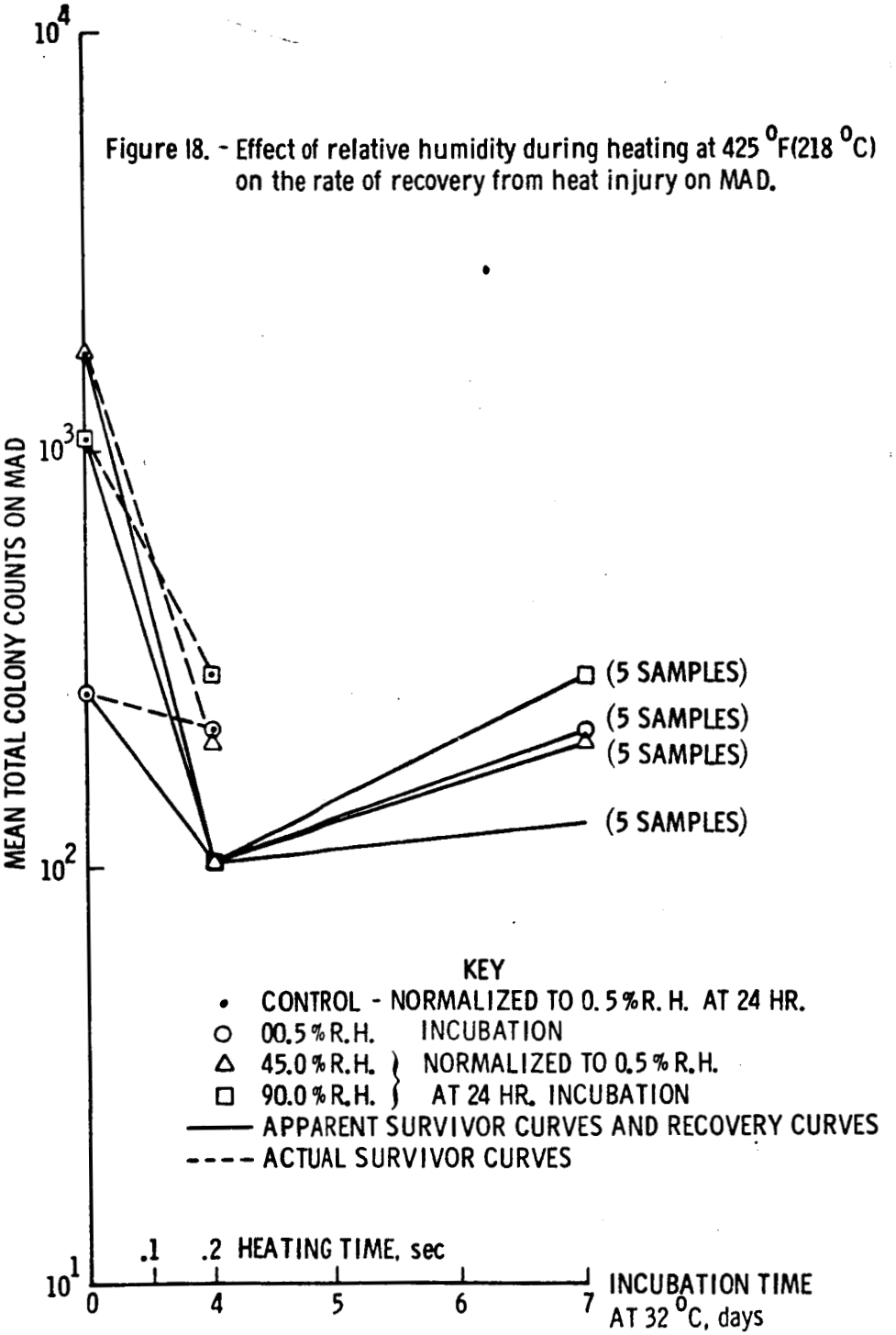

MEAN TOTAL COLONY COUNTS ON TSA
10^4
10^3
10^2
10^1
(4 SAMPLES)
(3 SAMPLES)
(3 SAMPLES)
(6 SAMPLES)
KEY
• CONTROL - NORMALIZED TO 0.5% R. H. AT 24 HR.
○ - 00.5 % R.H. INCUBATION
△ - 45.0 % R.H. } NORMALIZED TO 0.5 % R.H.
□ - 90.0 % R.H. } AT 24 HR. INCUBATION
APPARENT SURVIVOR CURVES AND RECOVERY CURVES
ACTUAL SURVIVOR CURVES
.1 .2 HEATING TIME, sec
0 1 2 3 4
INCUBATION TIME AT 32 °C, days

Figure 17. - Effect of relative humidity during heating at 425° F(218 °C) on the rate of recovery from heat injury on TSA.

Figure 18. - Effect of relative humidity during heating at 425 °F(218 °C) on the rate of recovery from heat injury on MAD.



In Figure 19 the effect of heat injury on D values at 90.0% R.H. can be observed. The base line is transposed directly from Figure 12 and was calculated from DELTA P's based on the survivors enumerated after 24 hours incubation on TSA at 32°C. The upper log D/temperature relationship was calculated from DELTA P's based on the survivors enumerated after 96 hours incubation on TSA at 32°C. The range of log D values at each temperature are presented, and the linear regression line was calculated in the same manner as for the earlier figures.

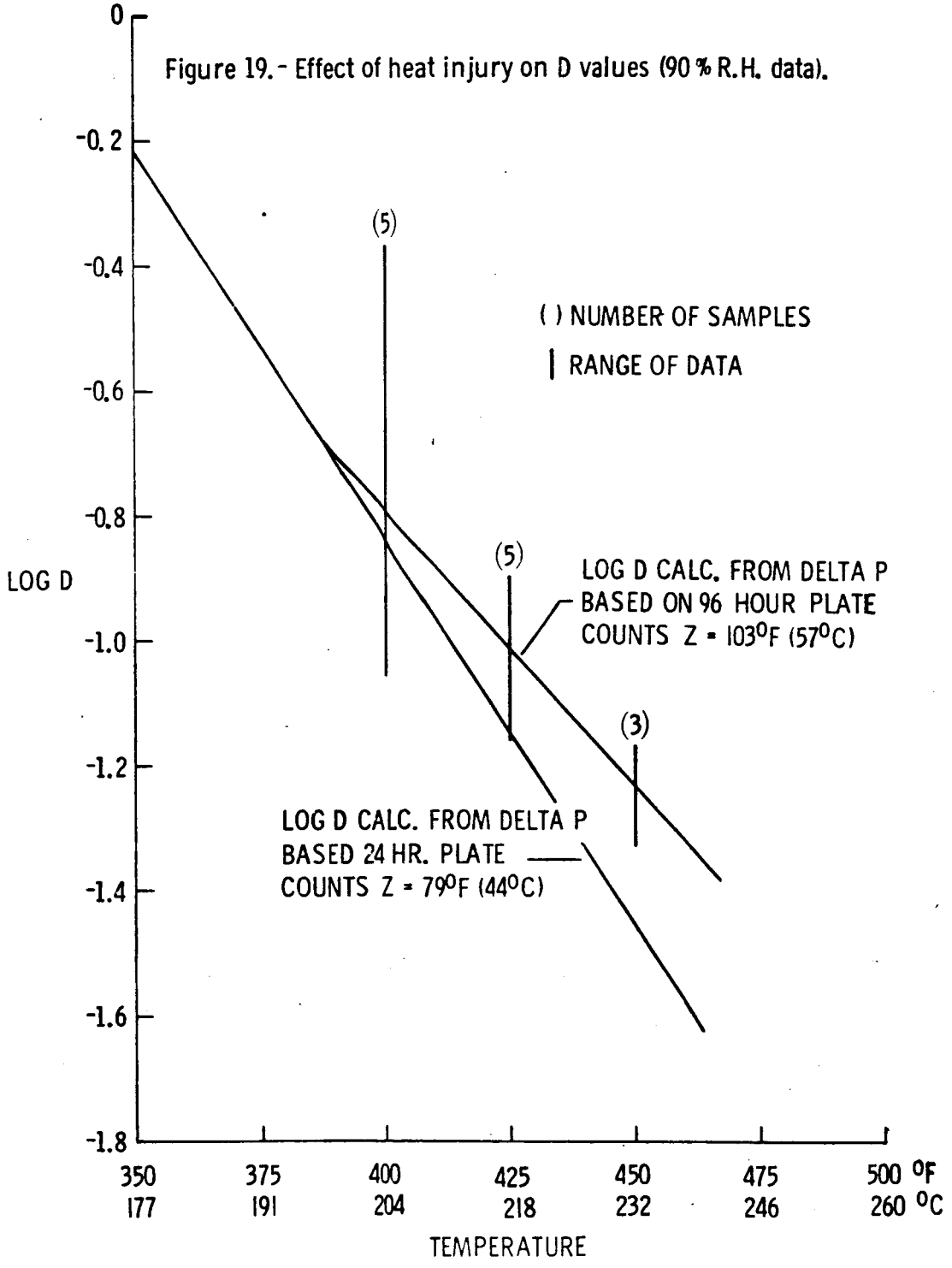


Figure 19.- Effect of heat injury on D values (90 % R.H. data).

DISCUSSION

A number of major observations can be made from the research data. These observations are discussed below with particular emphasis being placed on the effect of each phenomenon on the use of heat as a technique for the sterilization of gases. The sequence of topics is a logical progression and does not necessarily follow the data sequence presented in the RESULTS section, which is keyed to the order in which the experiments were performed.

A. D Values

The D values observed for the temperature range of 300-800°F (149-427°C) are generally not in agreement with previously published data nor is there much agreement within that body of data. This difference is very likely due to the wide variation in research techniques and systems observed among previous researchers and the differences between their techniques and systems and those used in this investigation. This relationship is demonstrated in Table 5 where specific D values from the log D/temperature relationships presented in Figure 13 can be compared with some representative D values from the literature. In order to minimize the effects of varying parameters, the data have been grouped according to three major parameters: (1) temperature, (2) ambient relative humidity or moisture level of spores, and (3) incinerator design. Although much effort has been expended, no logical interrelationships can be observed within these data and all attempts to explain differences on the basis

TABLE 5

Comparison of High Temperature D-Values*

Temperature °F / °C	300 / 250	350 / 177	400 / 200	450 / 232	500 / 260
Relative Humidity (Carrier gas)					
0.5(7)	0.20(7)	0.13(7)	.086(7)	.057(7)	.038(7)
"Dry" spores	(0.6)(1)	5.4(6)	(0.32)(1)		(.002)(5)
"Wet" spores	8.3(1)	.038(2)	4.1(1)	0.7-1.5(4)	0.18(8)
	(1.0)(1)	2.3-4.9(4)	(.26)(1) 1.13(3) 1.6-2.9(4)	(.002)(5)	0.55-1.2(4)
45.0(7)	0.78(7)	0.33(7)	0.14(7)	.058(7)	.025(7)

References

(1) Bourdillion et al. (1948)

(2) Gremillion et al. (1958)

(3) Elsworth et al. (1961)

(4) Chatigny et al. (1970)

(5) Mullican et al. (1971)

(6) Hoffman et al. (1968)

(7) This investigation.

(8) Barbeito et al. (1967)

*Internally heated systems proved values not in parentheses; those in parentheses are from externally heated systems; D is in seconds.

of experimental parameters other than those indicated here have been unsuccessful. Of the six comparable sets of data, only Gremillion et al. reported a D value smaller than those determined in this investigation.

The one abiding impression obtained from this analysis was that the incinerator design had the greatest impact on D values, although the effects of temperature and relative humidity were certainly noticeable if not consistent. Three incinerator design parameters appear to have the most effect on the observed differences in D values. First, the radiant heating of thermocouples causes internally heated incinerators to produce apparently lower D values because the thermocouples indicate a temperature higher than the true temperature of the flowing gas. Second, the effect of naked heating elements, other heating surfaces at temperatures considerably higher than that of the gas, and/or exposed flames is to produce kill which is not directly related to the gas temperature, thus producing apparently higher D values. Third, the flow rate apparently has some unknown effect on D values which is not directly related to the spore residence time. Investigators such as Decker et al. (1954) who observed a rather wide variation of flow rates obtained D values which were not directly proportional to temperature, but rather to a combination of temperature and flow rate. This effect may be related to temperature measurement inaccuracies caused by the gas flow. Thus these effects should be considered in the design of future incinerator systems, particularly those to be used for experimental purposes, and

in the use of data from internally heated experimental systems.

B. Z Values

The Z-values calculated from log D/temperature relationships observed in this investigation are basically in agreement with Z-values calculated from published data, which indicates that variations in research methods discussed above have much less effect on these values. The Z-values presented in Figure 13 are compared in Table 6 with some values presented in or calculated from data in the indicated references. A strong relationship between Z-value and relative humidity is observed which correlates very well with the relationship presented in Figure 14. Although the absolute humidities (not shown) may vary considerably, the slope of relationship is sufficiently slight to produce small variations of Z-value among researchers using similar temperatures and relative humidities. Note particularly the small difference in Z-value between wet spores at ambient (30-45% ?) relative humidity and dry spores at 45% R.H. and the large difference between the former data and dry spores at ambient relative humidity. This may be due to increased moisture levels produced by the wet nebulizer systems. Mullican et al. (1971) noted a 13% increase in carrier gas relative humidity due to this effect. Finally, comparisons of Z-values obtained at different relative humidities should be restricted to similar temperature ranges. The reason for this restriction will become apparent in subsequent discussions.

TABLE 6

Comparison of Z Values for Bacillus subtilis var. niger

Carrier Gas % Relative Humidity	Wet or Dry Spores	Temperature Range (°F)	Z Value (°F/°C)	Reference
0.5	Dry	300-550	278/154	This investigation.
Ambient[1]	Dry	300-390	333/185	Bourdillion et al. (1948)
Ambient[1]	Wet	210-390	185/103	Bourdillion et al. (1948)
Ambient[1]	Wet	270-470	165/92	Elsworth et al. (1961)
Ambient[1]	Wet	350-500	155/86	Chatigny et al. (1970)
45.0	Dry	400-500	133/74	This investigation.
90.0	Dry	400-450	74/41	This investigation.
100.0	Wet	255-275	24-33/ 13-18	Edwards et al. (1965a)[2]

[1]Assumed to be 30-45% relative humidity.
[2]Data for B. subtilis strain A.

C. Transition Points

The occurence of a transition point in Figures 10, 11, and 12 where the Z-value of the log D/temperature relationship increases very significantly is of obvious interest. The Z-values obtained for this highest temperature range are very large, being between 4000 and 9000°F, and are not apparently related to relative humidity. This leads to the conclusion that this portion of the relationship may be where log D is constant with increasing temperature. Since this relationship has not been reported as such in the literature, a particularly thorough discussion of the phenomenon seems warranted. First, it should be noted that Barbeito et al. (1968) presented data which indicated a Z-value of almost 4000°F for the temperature range of 525-625°F(274-329°C) using wet spores of B. subtilis var. niger, relative humidity unknown. These data fit the relationship observed in this study, but is not commented on by the authors in their discussion.

Since the location of the transition point is both temperature and relative humidity related, a fixed combination of these parameters may produce an unknown effect which is manifested in this manner. Although no specific mathematical/thermodynamic relationship has been identified, a possible theory has been considered and is presented to serve as a guide for subsequent experiment design and data analysis. The rate of heat transfer from the surface of the spore, which in internally heated incinerators is heated by both convection

and radiation, to the heat sensitive site within the spore is proportional to both temperature and the water activity (a_w) of the spore. Since the a_w of the spore is proportional to the relative humidity of the carrier gas, the above relationship also holds for relative humidity. Therefore, it is proposed that a maximum rate of heat transfer occurs within the spore which is a function of both temperature and the ambient relative humidity and which results in the observed maximum temperature effect. This relationship appears to be supported by the data, i.e. 90% R.H. would allow the maximum rate of heat transfer to occur at a lower temperature (460°F/238°C) than would 0.5% R.H., which required 550°F(288°C) to produce the maximum lethal effect.

Since the transition points at all relative humidities occur when the population has been reduced by approximately 99.99%, a second possible explanation for their occurance can be proposed. If the spore population being tested was dichotomous and the smaller fraction (0.01%) was extremely heat resistant, a similar type of transition in the time/temperature relationship would be noted. However, this seems unlikely in this case cause the very large Z-values observed are at least ten fold larger than those reported for even the most heat resistant sporeforming bacteria.

The occurence of another transition point may be indicated by the data presented in Figures 10 and 11. The increase in variation of log D noted at the high temperature transition point is also noted

at 300°F(149°C) at 0.5% R.H. and at 400°F(204°C) at 45.0% R.H., but not at the minimum temperature at 90% R.H. This increased variation may indicate the data are approaching another transition point. This would not be unexpected for two reasons. First, extrapolation of the plot of Bruch (1968) for dry B. subtilis var. niger spores on paper at temperatures between 200°F(93°C) and 285°F(140°C), Z equal to 49°F (27.2°C), to a higher temperature range produces an intersection with the 45% R.H. curve just below 400°F(204°C). Second, multi-Z-relationships have been reported at least twice in wet heat research (Esselen and Pflug, 1956 and Edwards et al., 1965a). Both of these investigations noted a small Z-value at the low end of their temperature range, and larger Z-values at the upper end. Actually some non-linearity of the log D/temperature relationship has to occur somewhere, as the Z should approach zero as lethality approaches zero at low temperatures.

The possible existance of two transition points within this temperature range poses the question as to whether the entire log D/temperature relationship might not be a curve rather than a sequence of linear functions. This possibly has been explored with the data presented in Figures 10-12. Hyperbolic functions were manually applied to the data but poor correlations were obtained.

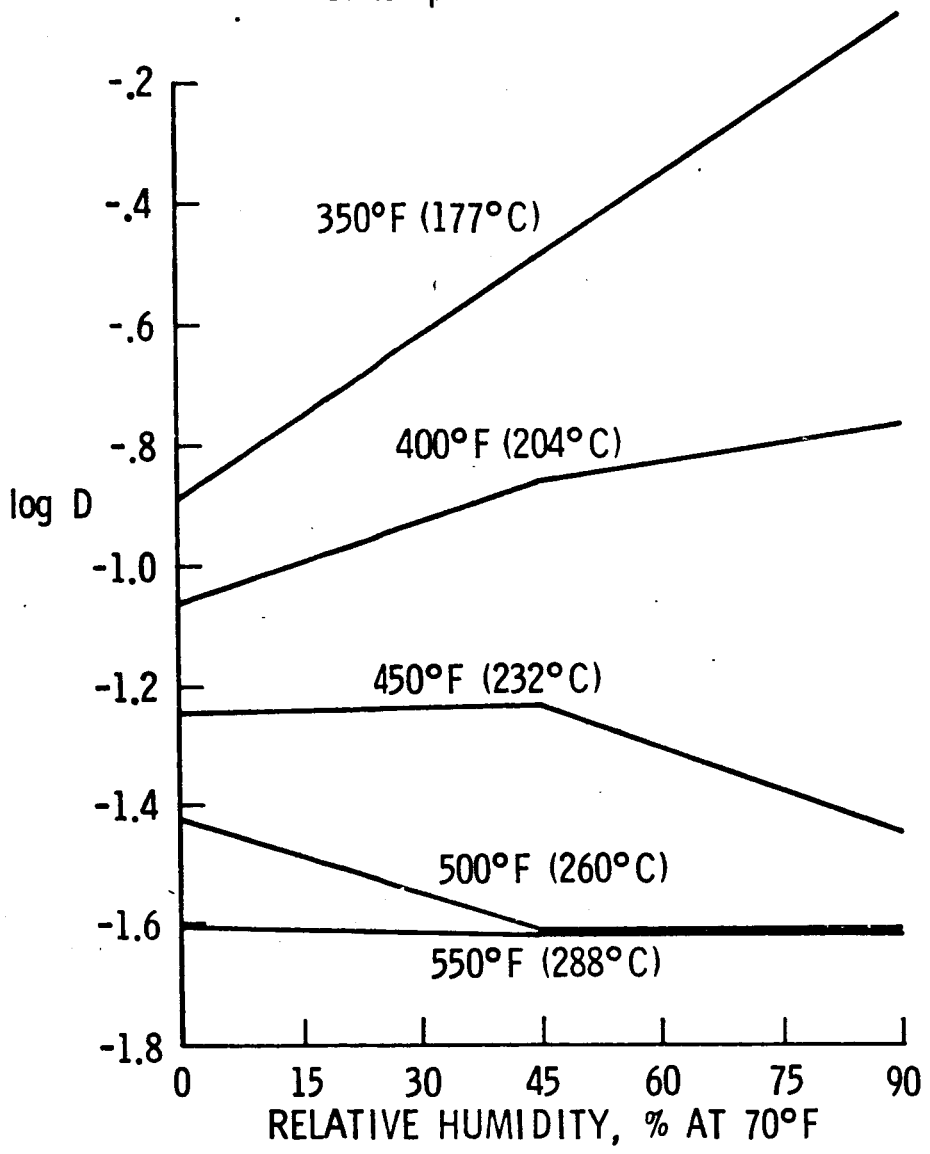
The occurences of transition points in the log D/temperature relationship (sometimes called the DRT, decimal reduction time, plot) has been proposed by Esselsen and Pflug (1956) to be caused by

chemical changes in the heating medium which occur at increased temperatures, and this could also be possible in the other case reported, but obviously not in this situation. It is much more likely that these changes in Z-value relate to changes in the mechanism of spore death (lethal injury).

D. Effects of Relative Humidity

The relationship of moisture level (R.H. or a_w) to D value is not consistent with increasing temperature, but instead passes through several distinct phases between 350 and 550°F(177-288°C). Reference to Figure 13 will quickly illustrate this phenomenon. From 350 to 400°F(204°C) the 90% R.H. has the lowest lethality, from 400 to 455°F (232°C) the 45% R.H. has the lowest lethality, and from 455 to 550°F (288°C) the 0.5% R.H. has the lowest lethality. Above 550°F the moisture level (R.H.) shows no significant effect on lethality. These relationships are perhaps shown more graphically in Figure 20. A plot of the corrected log D versus temperature (not shown) comes very close to producing a double "crossover" point, which eliminates the 45% R.H. portion and reduces the observed number of relationships to three. These observations could be explained by the predominance at low temperatures of the "loss of stabilizing water molecules" effect noted by Brannen (1971) at low moisture levels and the increasing predominance in the higher temperature range (above 450°F/232°C) of the "increase in water-bridges in macro-molecules" effect proposed by Gilbert et al. (1964) at high moisture levels. This sequence of

Figure 20 - Variation of log D with relative humidity as a function of temperature

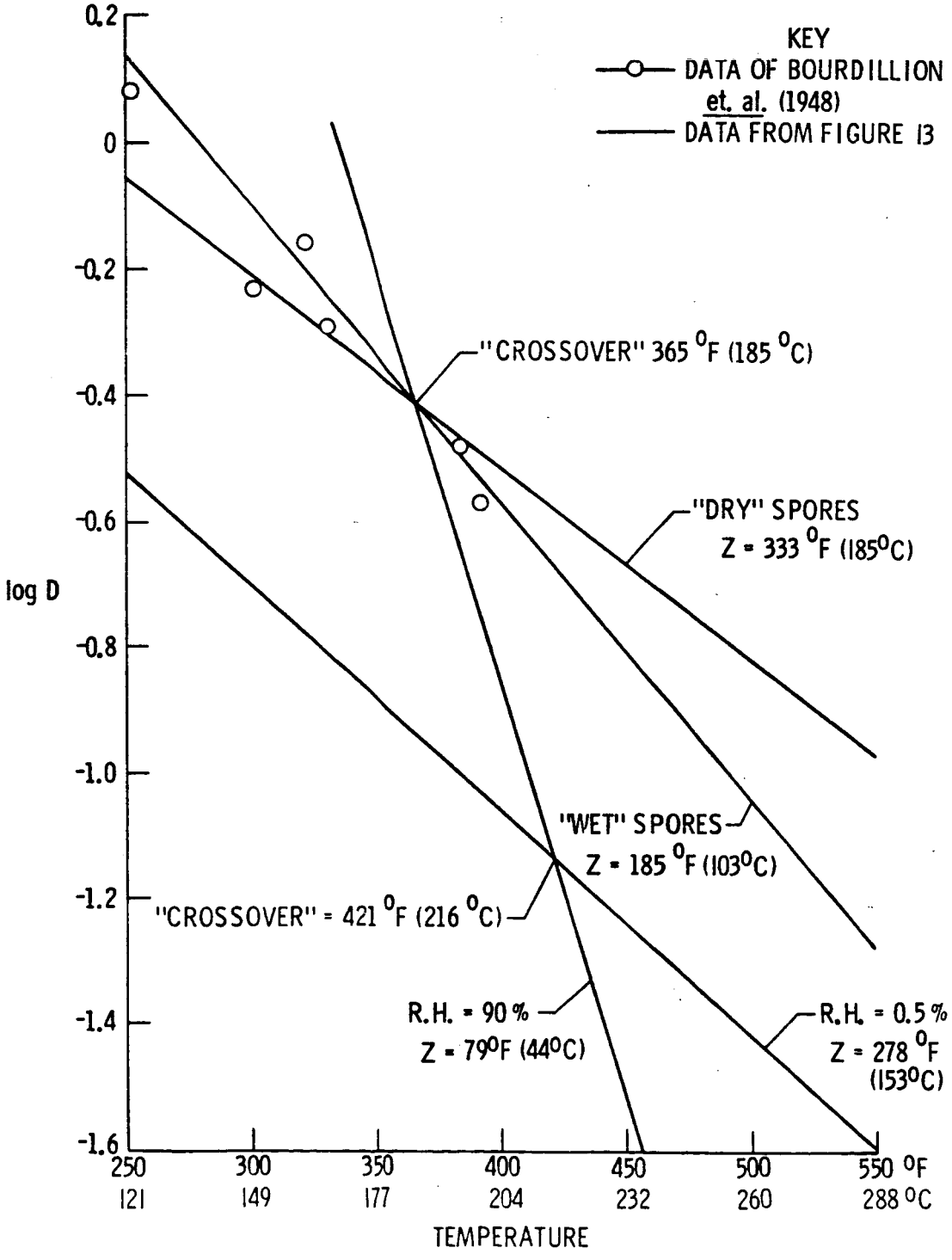


events may well be repeated at temperatures below 350°F(177°C), as the results of Murrell and Scott (1966) and Angelotti et al. (1968) between 212 and 275°F(135°C) closely approximate the effects noted in this investigation between 400 and 455°F(204-232°C), i.e. the point of lowest lethality is about 45% R.H. and trending toward lower values of relative humidity as temperature increases.

Two other reports of "crossover" in relative humidity and lethality at high temperatures have been reported. Bourdillion et al. (1948) reported data which indicates a "crossover" at 365°F(185°C) between the log D/temperature relationship determined with "dry" spores and that determined with "wet" spores. This relationship is compared in Figure 21 with that determined in this investigation at R.H. of 90 and 0.5 percent. Hoffman et al. (1968) reported data that indicate a double "crossover" similar to that noted with corrected data in this investigation, but their point was at 378°F(192°C). Their relative humidity values were 11, 33 and 85 percent. Considering the variability in D-value caused by variations in systems and techniques noted earlier in this discussion, and noting the differences in experimental systems used by the three investigators, the differences in crossover points (temperatures) noted in the three investigations is very likely within the combined experimental error.

The relationship of moisture content to Z-value observed in this investigation is evidently uniform throughout the experimental temperature range (Figure 14) and, as mentioned earlier in this discussion, is supported by most of the data found in the literature. This

Figure 21. - Comparison of D and Z values with data of Bourdillion et. al.



relationship may continue at higher relative humidities which would produce supersaturated conditions at 70°F(21°C).

E. Optimizing the Biological Efficiency of Incinerators

The biological efficiency of an incinerator system can be optimized by comparing the electrical power required to obtain a set temperature with the lethality (1/D) of that temperature. A plot of this relationship over the experimental temperature range is presented in Figure 16 which was previously introduced in the RESULTS section. The conclusion obtained from this plot is that, contrary to the early belief expressed in the INTRODUCTION to this dissertation, there is a limit to the concept that lethality increases exponentially while the power requirement increases linearly. This limit, the optimum operating temperature, is related to the relative humidity of the incoming gas and is the transition point for that humidity (See page 75). Above this temperature additional increases in temperature will produce little if any additional lethality.

F. Corrected D and Z Values

Contrary to a long held assumption, the heat-up and cool-down of the gas and the spore or natural aerosol in most incinerator systems produce significant reductions in the biological population, which when not subtracted from the total thermal effect, result in the calculation of the D-values for the "set point" temperatures which are artificially low. Dependence on these D-values in

sensitive applications areas could result in accidental contamination of products or environments. The significance of this correction is indicated in Figure 15. The correction for heat-up and cool-down effects produced 15.1, 23.9, and 32.1% increases in D-value at temperatures of 300, 400 and 500°F(149, 204 and 260°C) respectively at an ambient R.H. of 0.5%. Similar correction effects were noted at the other relative humidities. The primary value of corrected D values would be where the total lethality of a heat process or exposure is to be determined from D-values. The use of corrected D values based on B. subtilis var. niger would be sufficiently conservative for almost any "dry" heat process, if they were determined at equivalent relative humidities.

The correction for heat-up and cool-down also affects the Z-values. This effect is caused by the increased percent of the total heat exposure which occurs in these processes as the "set" temperature increases. In the example presented in Figure 15, the Z-value is increased by approximately 9% as a result of the correction process.

The agreement between the computer calculation of the correction and the manual calculation described earlier has been excellent, generally being within 0.02 seconds of agreement. Thus, this method of analysis should be applicable to other experimental and operational incinerator systems. The temperature data of a number of investigators (Chatigny et al., 1970, and Barbeito et al., 1967, for example) could easily be analysed by this program.

G. Sub-lethal Heat Injury Effects

The heat injury effects (sub-lethal) that were observed in this investigation are proportional to relative humidity and appear to be caused by inhibition of spore germination. The primary indication of the heat injury has been the delayed colony formation exhibited by heated organisms on both a complete (TSA) and a minimal (MAD) medium. Reference to Figure 17 provides evidence of the relative humidity relationship. A linear relationship between the normalized number of injured spores which recovered on TSA and relative humidity of the heated gas is observed, which produces greater numbers of injured spores as the relative humidity increases. Since this incinerator temperature (425°F/218°C) was selected for the experiments because the effect of relative humidity on the lethality of the heating process is slight, it appears that this relationship is in addition to the lethal effect, rather than an adjunct of it, i.e. different mechanisms may be involved. A similar relationship is noted between 45 and 90% R.H. when the number of injured spores which recovered on MAD are compared against relative humidity (Figure 18). However, below 45% R.H. there appears to be no such relationship.

Since higher counts of unheated B. subtilis var. niger spores are obtained when incubated at 32°C than at 45°C, it was not too surprising that the same relationship was noted with the heated spore population. Thus, although the same relationship was noted as was reported by Edwards et al. (1965b), for wet heat injured spores, it

did not constitute a reversal of the unheated situation as was reported by them for B. subtilis. However, the slight inhibitory effect of a minimal medium on the counts of heated B. subtilis var. niger directly paralleled the dry heat results of Augustin and Pflug (1967) with Putrefactive Anaerobe 3679.

The change in D-values indicated by the difference in the slope of the "apparent" and "actual" survivors curves presented in Figures 17 and 18 is more graphically represented in Figure 19 by the 90% R.H. data. Here a 37 and 68% increases in D-value are noted at 425 and 450°F(218 and 232°C) respectively due to the subtraction of the heat injury effect. Although these percentages are somewhat lower at lower relative humidities the differences still appear significant. These observations are very similar to those which can be made from the data of Bourdillion et al. (1948), which were observed using their internally heated incinerator. A comparison of data indicates that injury begins about 320°F(160°C) in their system and about 385°F (196°C) in this system, a difference which may be related to relative humidity, and increases with temperature until all of the survivors are injured. Therefore, any testing of experimental or operational incinerator systems should account for the heat injury effect by utilizing the longer incubation times and a complete medium such as TSA when enumerating survivors.

Some inferences as to the mechanism of the observed heat injury can be made from the data. The absence of any significant reduction

in the magnitude of heat injury observed when temperature and nutritional stresses were imposed on the survivors of the heating process indicates that the injury mechanism is more likely related to inhibition of germination than to the inability of germinated spores to produce outgrowth. The effects of relative humidity on the number of injured spores observed may further support this conclusion, since the water related effects proposed by Gilbert et al. (1964) relate more to macromolecules such as proteins than they do to macromolecules such as DNA and RNA. Also, Adams and Busta (1972) related their wet heat injury in Bacillus subtilis to protein denaturation. Since the spore lytic enzyme has been previously linked to wet heat injury in similar organisms (Hashimoto et al., 1972, Gould, 1970) this would seem to be a likely candidate for this effect.

Further studies in this area using some of the many techniques developed in recent years should be rewarding. Although use of lysozyme treatment is voided due to the sensitivity of the organism, other germination stimulating techniques should shed additional light on the mechanism of the observed heat injury.

SUMMARY AND CONCLUSIONS

Several interrelating studies have been performed to determine the lethal and sublethal effects of ultra-high temperatures (300-800°F/149-427°C) on Bacillus subtilis var. niger as a function of ambient relative humidity. First, time/temperature relationships required to obtain uniform reductions in spore populations of this organism were determined by a comparison of the number of survivors of air-borne aerosols passed through a heated and unheated electric incinerator. Incinerator temperatures were varied while the relative humidity was held constant at 0.5, 45.0, and 90.0 percent. At the low end of the temperature range a logarithmic relationship was found to exist between the exposure time required to obtain a 90 percent reduction in population (D-value) and temperature, but above certain critical, humidity related temperatures the relationship was observed to be constant at a D-value of about 0.031 seconds. The critical or transition temperatures were determined to be 460, 500, and 555°F(238, 260 and 291°C) for 90.0, 45.0, and 0.5 percent relative humidity respectively, indicating an inverse relationship between relative humidity and the temperature of the transition point. This was also found to be the relationship between the log of Z, an index of the slope of the log D/temperature relationship, and relative humidity. Values of Z for 0.5, 45.0 and 90 percent relative humidity

were determined to be 276, 133, and 79°F(153, 74, and 44°C) over a temperature range from 300 or 400°F(149 or 204°C) to the transition point. The relationship between moisture content (R.H. or water activity (a_w)) and D value was not consistent with increasing temperature, but was found to pass through several phases.

Analysis of the lethal effects of the heat-up and cool-down of the spore aerosol during these studies indicated that significant reductions in the populations were produced by these heat exposures and that allowances for these effects would produce significant increases in the D-values computed for the "set point" temperatures of the incinerator. A computerized analysis of temperature profiles has been specifically developed to make these corrections, which can be applied to other incinerator or dry heat oven data.

Using some of D-values obtained from the time/temperature studies, a relationship has been determined between the biological efficiency of an incinerator system and the electrical power required to obtain that efficiency. From this relationship and optimum operating temperature can be determined for any ambient relative humidity.

Finally, a study of the sub-lethal or heat injury effects of these temperatures and relative humidities on B. subtilis var. niger spores was made by changing the time/temperature procedures to allow enumeration of heat injured organisms. The primary indication of heat injury was delayed colony formation in the recovery media. The heat injury effects observed were proportional to relative humidity and appear to be caused by inhibition of spore germination.

Sufficient levels of heat injured spores were found to indicate that this effect should be considered whenever the biological efficiency of an incinerator system or a dry heat oven is being evaluated.

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```
      PROGRAM HEATER (INPUT,OUTPUT,TAPE5=INPUT,TAPE6=OUTPUT)
      DIMENSION TIME(6), TEMP(6), NT(7), TMAX(100), AREA(100), DMIN(100)
     1,LAB(2), ERR(5,5), T2(100), T5(100), AREAI(100) , DMAX(100)
      DATA T/391.,400.,425.,450.,600.,800.,900.,1000./
      DATA TMIN/391./
      DATA DTL/9.,2*25.,150.,200.,2*100./
      DATA NT/7*0/
      DIMENSION DTC(100)
      DIMENSION DINV(7),X(7,100),T(8),DTL(7)
      COMMON/LINK1/A1(100),B1(100),C1(100),D1(100),A2(100),B2(100),C2(10
     10), D2(100)
      COMMON/LINK2/ B,U,V
      DATA TIME/-.078,-.039,0.,0.,.0117,.0312/
      DATA DTC0/.078/
      CALL PSEUDO
      N=0
      READ(5,410) LABEL, TCO
  410 FORMAT(A10, F4.0)
      READ(5,411)IDENT,TEMP(3),TEMP(2),TEMP(1),TEMP(4),TEMP(5),TEMP(6),D
     1ELTAP,VDOT
  411 FORMAT(16X,A10,5X,3(F3.0,2X)/31X,3(F3.0,2X)/F5.2,1X,F5.2)
      INDX=1
      LAB(1)=10H FHF B1250
      LAB(2)=LABEL
      PRINT 400, LABEL
  400 FORMAT(1H1* SUMMARY OF *A10* DATA*/*  N    IDENT     DELTA P*44X*TI
     1ME/TEMP/COEFFICIENTS*/35X*A1*10X*B1*10X*C1*10X*D1*6X*MAX T*6X*C2*1
     20X*D2*10X*A2*10X*B2*//)
      GO TO 2
    1 READ(5,411)IDENT,TEMP(3),TEMP(2),TEMP(1),TEMP(4),TEMP(5),TEMP(6),D
     1ELTAP,VDOT
      IF(EOF,5)25,2
    2 N=N+1
  402 FORMAT(I4,1X,A10,E11.3,F7.3,2(16X,F7.3),F7.3,2(16X,F7.3)/26X,F7.1,
     12(16X,F7.1),F7.1,2(16X,F7.1))
```

```
      DO 201 I=1,6
  201 TIME(I)=TIME(I)/VDOT
      DTC(N)=DTC0/VDOT
      TMAX(N)=(TEMP(3)+TEMP(4))/2.
      AREAI(N)=0.
      AREA(N)=DELTAP
      D1(N)=ALOG(TMAX(N))
      D2(N)=D1(N)
      C1(N)=(ALOG(TEMP(2))-D1(N))/(TIME(2)**2)
      C2(N)=(ALOG(TEMP(5))-D2(N))/(TIME(5)**2)
      B1(N)=(ALOG(TEMP(2))-ALOG(TEMP(1)))/(TIME(2)-TIME(1))
      B2(N)=(ALOG(TEMP(6))-ALOG(TEMP(5)))/(TIME(6)-TIME(5))
      A1(N)=ALOG(TEMP(2))-B1(N)*TIME(2)
      A2(N)=ALOG(TEMP(5))-B2(N)*TIME(5)
      T2(N)=TEMP(2)
      T5(N)=TEMP(5)
   20 PRINT 402,N,IDENT,DELTAP,TIME,TEMP
      PRINT 413, A1(N),B1(N),C1(N),D1(N),TMAX(N),C2(N),D2(N),A2(N),B2(N)
  413 FORMAT(29X,4E12.4,F7.1,4E12.4)
      DO 21 I=1,6
   21 TIME(I)=TIME(I)*VDOT
      GO TO 1
   25 NBR=N
      NMAX=NBR
      DO 26 I=1,700
   26 X(I)=0.
      DO 40 N=1,NBR
      J=(TMAX(N)-387.5)/25.+1.
      IF(J.LT.9) IMAX=J+1
      IF(J.EQ.9) IMAX=5
      IF(J.GT.9) IMAX=6
   30 IM1=IMAX-1
      NT(IM1)=NT(IM1)+1
      TL=TMIN
      DO 31 I=1,IM1
      CALL TINVERT(TL,THU,TCD,N,T2(N),T5(N))
```

```
      T(I)=.5*(TCD-THU)
   31 TL=TL+DTL(I)
      T(IMAX)=0.
      IM2=IM1-1
      IF(IM2) 40,40,32
   32 DO 33 I=1,IM2
   33 X(I,N)=T(I)-T(I+2)
   40 X(IM1,N)=DTC(N)+T(IM1)
      IMAX=IM1
      PRINT 440, LABEL,TMIN
  440 FORMAT(1H1,*   DATA IDENTIFICATION *A10/
     13X*DELTA P =      1/D(300) +    1/D(400) +    1/D(500) +    1/D(55
     20) +    1/D(600) +    1/D(700) +    1/D(800)*//*  1/D(*F3.0*) = 0.
     3*//)
      N2=NT(1)
      I=1
      T(1)=TMIN+DTL(1)
      DINV(1)=0.
      DO 41 N=1,N2
      PRINT 441, AREA(N),X(1,N)
   41 DINV(1)=DINV(1)+AREA(N)/X(1,N)
      DINV(1)=DINV(1)/NT(1)
      PRINT 442, T(1),DINV(1)
   42 IM1=I
      I=I+1
      N1=N2+1
      N2=N2+NT(I)
      T(I)=T(I-1)+DTL(I)
      DINV(I)=0.
      DO 45 N=N1,N2
      PRINT 441, AREA(N),(X(J,N),J=1,I)
      SUM=AREA(N)
      DO 44 J=1,IM1
   44 SUM=SUM-DINV(J)*X(J,N)
```

```
 45 DINV(I)=DINV(I)+SUM/X(I,N)
    DINV(I)=DINV(I)/NT(I)
    PRINT 442, T(I),DINV(I)
    IF(I.LT.IMAX) GO TO 42
441 FORMAT(F10.2,2X,7E14.4)
442 FORMAT(  //*  1/D(*F3.0*) = *E12.4//)
    XAX=10H    TEMP
    YAX=10H    1/D
    I=IMAX
    CALL VDIPLT(1,LAB,I,T,DINV,100.,900.,0.,0.,-1,XAX,-1,YAX,2)
    PRINT 470, LABEL
470 FORMAT(1H1,1X,A10* PREDICTED POPULATION REDUCTIONS USING BEST FIT
   1COEFFICIENTS*/*   N*6X*MAX T*6X*ACTUAL DELTA P*4X*COMPUTED DELTA P
   2*10X*DIFFERENCE PERCENT DIFFERENCE*6X*AC*10X*AW*12X*PCT*//)
    DO 71 N=1,NBR
    AREAI(N)=0.
    DO 70 I=1,IMAX
 70 AREAI(N)=AREAI(N)+X(I,N)*DINV(I)
    DIF=AREA(N)-AREAI(N)
    PCDIF=DIF/AREA(N)*100.
    J=(TMAX(N)-387.5)/25.+1.
    IF(J.EQ.9) J=4
    IF(J.GT.9) J=5
    AREAC=DTC(N)*DINV(J)
    AREAW = AREAI(N)-AREAC
    PCTW = 100.*AREAW/AREAI(N)
 71 PRINT 471,N,TMAX(N),AREA(N),AREAI(N),DIF,PCDIF, AREAC, AREAW, PCTW
471 FORMAT(I4,4X,F7.1,3(8X,E12.4),10X,F10.3, 2(E12.4),3X,F10.3)
    YAX=10H LOG(1/D)
    CLOG=ALOG(10.)
    DO 38 I=1,IMAX
    IF(DINV(I).LE.0.) DINV(I)=1.E-4
 38 DINV(I)=ALOG(DINV(I))/CLOG
    PRINT 438, LABEL,(T(I),DINV(I),I=1,IMAX)
438 FORMAT(1H1*  DATA IDENTIFICATION*2X,A10//*     TEMP       LOG(1/D)*/
   1/ (F8.0,4X,E12.4))
```

```
      I=IMAX
      CALL VDIPLT(1,LAB,I,T,DINV,100.,900.,0.,0.,-1,XAX,-1,YAX,14)
      CALL CALPLT (0.0,0.0,999)
      STOP
      END
      SUBROUTINE TINVERT(T,THU,TCD,N,T2,T5)
      COMMON/LINK1/A1(100),B1(100),C1(100),D1(100),A2(100),B2(100),C2(10
     10),D2(100)
      IF (T-T2) 1,1,2
    1 THU=(ALOG(T)-A1(N))/B1(N)
      GO TO 3
    2 THU2=(ALOG(T)-D1(N))/C1(N)
      IF(THU2.LT.0.) THU2=0.
      THU=-SQRT(THU2)
    3 IF (T-T5) 4,4,5
    4 TCD=(ALOG(T)-A2(N))/B2(N)
      RETURN
    5 TCD2=(ALOG(T)-D2(N))/C2(N)
      IF(TCD2.LT.0.) TCD2=0.
      TCD=SQRT(TCD2)
      RETURN
      END
```

VITA

Franklin Harris Farmer was born in [redacted] on [redacted] to Franklin and Kora Temple Farmer. He was raised in Portland and Falmouth, Maine and graduated from Falmouth High School in 1956. After graduating from Tufts University in 1960 with a Bachelor of Science degree in Biology, he was commissioned an ensign in the U. S. Naval Reserve and spent a two year tour of active duty at the U. S. Naval Amphibious Base, Little Creek, Norfolk, Virginia as a communications officer. In 1962, he married Janice Joyce Howard of Portsmouth, Virginia. The Farmers now have three children.

In 1962, the author entered the Graduate School at Virginia Polytechnic Institute (now State University) and completed the requirements for a Master of Science degree in Bacteriology in March, 1965. In April of that year he was employed by the National Aeronautics and Space Administration's Langley Research Center as microbiologist. He completed 18 months of graduate study at the University of Minnesota during 1967-68. Finally, in 1972, he returned to VPI&SU to begin work on the Doctor of Philosophy degree in Food Science and Technology, completing his research "in absentia" at NASA/LRC during the last two years.

EFFECTS OF ULTRA-HIGH TEMPERATURES ON BACILLUS SUBTILIS VAR. NIGER SPORE AEROSOLS AS A FUNCTION OF RELATIVE HUMIDITY

by

Franklin Harris Farmer

(ABSTRACT)

The lethal and sub-lethal effects of ultra-high temperatures (300-800°F/149-427°C) on Bacillus subtilis var. niger spores as a function of ambient relative humidity were determined by a comparison of the number of survivors of air-borne aerosols passed through a heated and un-heated electric incinerator. Incinerator temperatures were varied while the relative humidity was held constant at 0.5, 45.0, and 90.0 percent.

At the low end of the temperature range a logarithmic relationship was found between the time required to obtain a 90 percent reduction in spore population (D value) and temperature, but above certain critical, humidity related temperatures the relationship was observed to be constant at a D value of about 0.031 seconds. The inverse relationship found between relative humidity and the critical transition temperature was also noted between relative humidity and the log of Z, an index of the log D/temperature relationship. Analysis of the lethal effects observed at these temperatures and relative humidities indicated that

correction for the lethal effects of heat-up and cool-down on the spore aerosol resulted in significantly higher D values, and that at any given ambient relative humidity an operating temperature can be determined for an incinerator which can provide an optimum combination of biological efficiency and electrical economy.

Using delayed colony formation on both a complete and minimal recovery medium as an indication of sub-lethal or heat injury effects, the effects observed were proportional to relative humidity, appeared to be caused by inhibition of spore germination, and represented significant changes in D value.